

Department of Applied Chemistry

**Characterisation of Polar Unresolved Complex Mixtures in
Groundwater Associated with Weathered Petroleum**

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Doctor of Philosophy
of
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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature:

Date:

“What matters most is how well you walk through the fire”

Charles Bukowski

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List of abbreviations

ARO	Aromatic fraction
Bgs	Below grounds surface
BHT	Butylated hydroxytoluene
BOC	Biogenic organic compound
BTEX	Benzene, toluene, ethylbenzene and xylenes
DBF	Dibenzofuran
DCM	Dichloromethane
DMA	Dimethyladamantane
DMI	Dimethylindanone
DMN	Dimethylnaphthalene
DMBF	Dimethylbenzofuran
DPM	Diphenylmethane
CoA	Coenzyme A
FID	Flame ionising detector
FTIR	Fourier transform infrared
GC	Gas chromatography
GW	Groundwater
MA	Methyladamantane
MBP	Methylbiphenyl
MI	Methylindanone
MDA	Methyldiamantane
MDBF	Methyldibenzofuran
MDPM	Methyldiphenylmethane
MS	Mass spectrometry
MSD	Mass selective detector
MSSV	Micro-scale sealed vessel
MT	Methyltetralone
NAPL	Non-aqueous phase liquid
NMR	Nuclear magnetic resonance
NOM	Natural organic matter
NVOA	Non-volatile organic acid
PAH	Polycyclic aromatic hydrocarbon

POL	Polar fraction
SAA	Combined saturate and aromatic fraction
SSM	Slow-stirring method
SW	Surface water
STD	Surrogate standard
TIC	Total ion chromatogram
TMA	Trimethyladamantane
TMB	Trimethylbenzene
TMN	Trimethylnaphthalene
ToF	Time of flight
TOC	Total organic carbon
TeMA	Tetramethyladamantane
TeMN	Tetramethylnaphthalene
TPH	Total petroleum hydrocarbons
TPHCWG	Total petroleum hydrocarbon criteria working group
UCM	Unresolved complex mixture
WD	Weathered diesel
VOA	Volatile organics analysis

Summary

Polar unresolved complex mixtures (UCMs) associated with weathered petroleum in groundwater have received little attention in the literature. These compounds can be incorporated in analytical techniques that are commonly used to measure the level of petroleum contamination in groundwater, such as total petroleum hydrocarbon (TPH) analyses. Distinguishing between polar compounds associated with weathered petroleum and natural organic matter (NOM) can be difficult, and may lead to TPH concentrations in groundwater that are not representative of the actual level of petroleum contamination. This thesis provides a detailed study of the dissolution, accumulation and formation of polar UCMs associated with weathered diesel. Novel techniques for differentiating polar UCMs sourced from petroleum and NOM were also developed.

Chapter 2 identifies the presence of polar UCMs in groundwater associated with weathered diesel. A polar compound (1-adamantanol) that can only be sourced from petroleum was identified suggesting the polar UCMs were derived from petroleum and not NOM. In Chapters 3 and 4, the formation, accumulation and dissolution of polar UCMs and related compounds from the biodegradation of petroleum were studied. These compounds were shown to accumulate in the weathered diesels and partition strongly into water (up to 96 mg/L) resulting in a similar polar contamination to that observed in the groundwater associated with weathered diesel in Chapter 2. Comparisons of the polar compounds identified in weathered diesel and associated groundwater were used to suggest where the polar compounds (and polar UCMs) form and accumulate (i.e. within the groundwater or the soil profile). These accumulation and dissolution processes for polar UCMs associated with weathered petroleum were suggested to be an ongoing source of persistent petroleum contamination and a potential risk to the environment and human health that can be misidentified or overlooked as NOM.

In Chapters 5 and 6, novel techniques for differentiating polar UCMs from petroleum and NOM in TPH were developed. The technique developed in

Chapter 5 differentiates polar UCMs present in TPH based on the differences in the carbon skeletons of petroleum and NOM. The technique applies a dehydrogenation reaction to reduce the complexity and compound types present in polar UCMs by defunctionalising and aromatising polar compounds into predominately hydrocarbons. Application of the technique to environmental samples containing polar UCMs from petroleum and NOM (some containing no identifiable hydrocarbons or polar compounds) showed the technique can be used to distinguish the sources of polar UCMs. The formation of diamondoid reaction products with carbon skeletons that can only be sourced from petroleum provided the most significant evidence for differentiating NOM and petroleum. The reaction products formed from petroleum polar UCMs also provided insight into the composition and formation of polar UCMs associated with weathered diesel. In Chapter 6, a simple screening tool was developed to allow the rapid assessment of the likely sources (NOM or petroleum) of polar UCMs present in surface water and groundwater. Assessment of the likely sources was based on differences in the TPH and TOC characteristics of NOM and petroleum polar UCMs.

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Chapter 1

1 Introduction

Petroleum contamination is an ongoing concern wherever petroleum is refined, stored or transported. Risk assessment of these events is often dictated by regulatory guidelines set for individual compounds and parameters that are largely based on abundant fresh petroleum components (petroleum hydrocarbons). Weathering processes can significantly change the composition of petroleum after its release and result in the formation of polar compounds. Current risk assessments associated with weathered petroleum are not aimed at measuring polar compounds, though they are often unavoidably incorporated. The presence of these polar compounds as an unresolved complex mixture (UCM) makes their differentiation from background natural organic matter (NOM) difficult. This chapter provides an overview of petroleum weathering in the environment, highlighting the difficulties involved in the assessment of environmental samples associated with weathered petroleum.

1.1 Petroleum contamination

Releases of petroleum are the most common source of persistent contamination in the environment (Kaplan *et al.*, 1997). The most highly publicised releases often occur in marine environments from oil tankers (Button *et al.*, 1992; Bence *et al.*, 1996) and oil wells (Adcroft *et al.*, 2010) with petroleum forming slicks on water surfaces, and tars that cover beaches (Bence *et al.*, 1996). Large volume terrestrial releases of petroleum from pipelines (Cozzarelli *et al.*, 1990; Baedecker *et al.*, 1993) and storage tanks (Chang and Lin, 2006) are common, though less frequently brought to public attention. These releases often result in soil saturation and the subsequent migration of petroleum as a non-aqueous phase liquid (NAPL) within the

subsurface (Kaplan *et al.*, 1997; Chen *et al.*, 2001; Johnston *et al.*, 2007). In these instances petroleum NAPLs that are less dense than water can accumulate and persist on top of the water table (Fig. 1.1), leading to long term contamination of soil (Molins *et al.*, 2010) and groundwater (Chen *et al.*, 2001; Hamed, 2004; Johnston *et al.*, 2007). This can be detrimental to the surrounding environment and potentially human health through the dissolution of toxic water soluble components into drinking water aquifers (Borden and Kao, 1992; Pearson and Oudijk, 1993; Wang *et al.*, 2002).

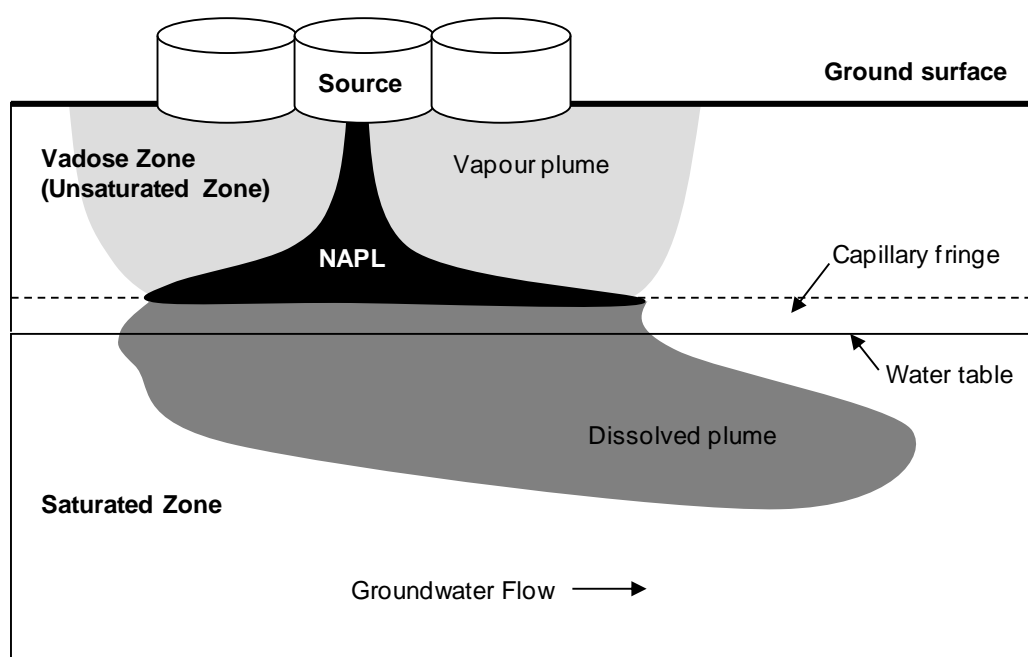


Figure 1.1 Volatilisation and dissolution of petroleum components in a NAPL contaminated subsurface environment.

1.2 Weathering of petroleum

Petroleum released into the environment typically undergoes changes as a result of physical, chemical and biological weathering processes (Christensen and Larsen, 1993; Kaplan *et al.*, 1996; Kaplan *et al.*, 1997; Kaplan, 2003). These processes result in the volatilisation, dissolution and degradation of petroleum components, occurring naturally (Barker *et al.*, 1987; Tiehm and Schulze, 2003; Lundegard and Johnson, 2006), or

enhanced through human intervention (Borden and Kao, 1992; U.S.EPA, 1999; Chaîneau *et al.*, 2003).

Weathering processes result in characteristic changes to the chemical composition of petroleum after its release. These changes have been well established for petroleum, and can be used to identify dominant weathering processes based on differences in compound susceptibilities to each process (Christensen and Larsen, 1993; Kaplan *et al.*, 1996; Kaplan *et al.*, 1997; Schmidt *et al.*, 2002a; Schmidt *et al.*, 2003). Interpretation of these compositional changes can give an estimation of a product's release date (Kaplan, 2003).

1.2.1 Volatilisation

Volatilisation is a significant weathering process that affects the volatile components of petroleum after its release (Kaplan *et al.*, 1996; Kaplan *et al.*, 1997; Kaplan, 2003; Kim and Corapcioglu, 2003). The extent a compound volatilises is dependant on its vapour pressure and concentration in the product (Christensen and Larsen, 1993). Light petroleum products such as gasoline contain a higher proportion of volatile components compared to middle-heavy petroleum distillates (e.g. diesel-lubricating oils) and therefore experience greater evaporative losses (Hoeppel *et al.*, 1991).

Volatilisation within the subsurface is the primary mechanism for the partitioning of volatile components from the NAPL into the soil gas phase (Kim and Corapcioglu, 2003). Once volatilised, components migrate through the vadose zone (unsaturated zone) as a vapour plume (Fig. 1.1) via diffusion and advection processes, leading to the long-term contamination of soil (Zaidel and Russo, 1993) and groundwater (Kim and Corapcioglu, 2003).

Volatilisation processes remove compounds with the lowest molecular weight and the highest vapour pressure (Kaplan *et al.*, 1996). Gas chromatography (GC) profiles of petroleum products that have undergone significant volatilisation will generally show a loss of the early eluting components (Kaplan *et al.*, 1996).

The relative amounts of specific compounds with different susceptibilities to volatilisation can be used to estimate the degree of alteration by volatilisation processes. These include *n*-pentane/*n*-heptane and 2-methylpentane/2-methylheptane, where lower values indicate evaporative losses due to the latter mentioned compounds being less volatile (Kaplan *et al.*, 1996).

1.2.2 Dissolution

Dissolution is a significant weathering process that affects water soluble petroleum components (Mackay *et al.*, 1991; Kaplan *et al.*, 1996; Schluep *et al.*, 2001). Within the vadose zone, water soluble petroleum components can be dissolved in pore water (de Blanc *et al.*, 1996) or infiltrating waters (Zalidis *et al.*, 1991; Annable *et al.*, 1993). NAPL accumulated on top of the capillary fringe above the water table (Fig. 1.1) can partition water soluble components across the NAPL/water interface and into the water phase as a result of concentration gradients (Mackay *et al.*, 1991; de Blanc *et al.*, 1996; Kim and Corapcioglu, 2003; Damera and Bhandari, 2007). Partitioning between NAPL and groundwater often results in a dissolved plume of petroleum components that migrates down-gradient (Fig. 1.1), with the most water soluble components found the greatest distance from the NAPL (Kim and Corapcioglu, 2003). The amount an individual component will partition into water is dependant upon several factors, including the compounds aqueous solubility, mole fraction (relative concentrations within the NAPL and groundwater) and the density of the NAPL (Lee *et al.*, 1992; Peters *et al.*, 1999).

Compositionally, dissolution processes initially remove the most water soluble components, such as low molecular weight aromatic hydrocarbons (i.e. benzene, toluene, ethylbenzene and xylenes; BTEX) (Kaplan *et al.*, 1996). Aliphatic hydrocarbons have very low water solubilities compared to aromatic hydrocarbons and these solubilities rapidly decrease with increasing molecular weight. For example, the water solubilities of *n*-octane, *n*-undecane and *n*-pentadecane are 660 µg/L, 9 µg/L, and 0.01 µg/L respectively (Tolls *et al.*, 2002). As a result, semi-volatile aliphatic

hydrocarbons are largely unaffected by dissolution processes. Aromatic hydrocarbon water solubilities also decrease with increasing molecular weight. For example, the water solubilities of benzene, naphthalene, and phenanthrene are 178000, 3100, and 110 µg/L respectively (CRC, 2007-2008).

The relative amounts of specific aliphatic and aromatic hydrocarbons with different susceptibilities to dissolution have been used to estimate the degree of alteration by dissolution processes. These compounds have similar susceptibilities to volatilisation and biodegradation so that changes in their relative abundances are largely a result of dissolution processes. Ratios used to assess these changes include benzene/cyclohexane and toluene/methylcyclohexane, where lower values suggest losses through dissolution (Kaplan *et al.*, 1996).

The dissolution of water soluble components from petroleum can be studied through “shake flask” and “slow stirring” dissolution experiments (Shaw and Reidy, 1979; Schluep *et al.*, 2001). Shake flask experiments involve the turbulent mixing of a NAPL and water by vigorous shaking in a separatory funnel. The disadvantage of this method is that micro-droplets can be formed in the water phase which can result in the inclusion of components with very low water solubilities. This causes a misrepresentation of the water soluble components, even to the extent that the profile of compounds analysed resembles that present in the NAPL phase (Shaw and Reidy, 1979; Schluep *et al.*, 2001). The slow-stirring method (SSM) promotes non-turbulent equilibrium of NAPL and water phases, keeping the NAPL/water interface intact and reducing the potential for micro-droplet formation (de Bruijn *et al.*, 1989; Schluep *et al.*, 2001). These dissolution experiments result in the partitioning of water soluble components from the NAPL into the water phase until equilibrium between both phases is reached (de Bruijn *et al.*, 1989). At this point, the measurement of a component's concentration in each phase gives rise to liquid-liquid partition coefficients that can be used to model the migration of dissolved organic compounds in groundwater (de Bruijn *et al.*, 1989; Lee *et al.*, 1992; de Blanc *et al.*, 1996).

1.2.3 Abiotic degradation

Abiotic degradation of organic compounds occurs through chemically and physically mediated reactions such as hydrolysis, elimination, chemical oxidation, and photo-oxidation (Hinman, 2003; Pierzynski *et al.*, 2005). Abiotic processes are generally considered insignificant in the degradation of petroleum hydrocarbons in soil (Bossart and Bartha, 1984) and groundwater (Capel, 1993; Borden, 1994), though biodegradation products of some non-hydrocarbon petroleum constituents may further degrade through hydrolysis (Laborde and Gibson, 1977). It has also been suggested that some sulfur containing non-hydrocarbon components such as thiophenes may undergo autooxidation in shallow reservoirs from exposure to oxic water (Waldo *et al.*, 1991), and this has been suggested as a possible mechanism for the degradation of some sulfur containing non-hydrocarbons present in crude oil contaminated groundwater aquifers (Thorn and Aiken, 1998).

Photochemical degradation (e.g. photo-oxidation) of petroleum has been well documented (Burwood and Spears, 1974; Hansen, 1975; Thominet and Verdu, 1984; Payne and Phillips, 1985; Burns, 1993; Charrie-Duhaut *et al.*, 2000; Al-Lihaibi, 2003). These reactions involve light mediated transformations to form oxidised products, which generally occur in surface water and are largely documented from petroleum spills in marine environments (Ali *et al.*, 1995; Guiliano *et al.*, 1997; Al-Lihaibi, 2003).

1.2.4 Biodegradation

Biodegradation is essentially a microbially driven redox reaction where a hydrocarbon is oxidised and an available electron acceptor is reduced (Borden, 2001). The process can occur aerobically, where oxygen is utilised as an electron acceptor (Leahy and Colwell, 1990) and anaerobically where alternate electron acceptors (i.e. nitrate, manganese (IV), iron(III), sulfate and carbon dioxide) may be utilised (Beller *et al.*, 1992; Bregnard *et al.*, 1996; Coates *et al.*, 1996; Heider *et al.*, 1998; Borden, 2001). During these

processes, microorganisms are able to utilise the energy from the oxidation of an organic compound to support growth (Fritzsche and Hofrichter, 1999). Oxidation can also occur without supporting microbial growth through a process known as co-metabolism (Horvath, 1972; Fritzsche and Hofrichter, 1999).

1.2.4.1 Biodegradation in the subsurface

Large volume releases of petroleum that migrate downward as a NAPL through the vadose zone and accumulate on top of the water table are subject to naturally occurring biodegradation processes. These processes occur throughout the soil profile of the vadose zone, and within groundwater of the saturated zone. It is generally accepted that microorganisms are only active in water (e.g. pore water), and biodegradation of petroleum components is therefore likely to occur within the water phase (de Blanc *et al.*, 1996) although several mechanisms for the uptake of hydrophobic petroleum components from NAPLs have been proposed (Fritzsche and Hofrichter, 1999; ITRC, 2009).

Vadose zone

Biodegradation within the vadose zone is well documented (Kristensen *et al.*; Davis *et al.*, 1998; Stout and Lundegard, 1998; Hers *et al.*, 2000; Holden and Fierer, 2005; Davis *et al.*, 2009; Molins *et al.*, 2010) and likely carried out by microorganisms within pore water (de Blanc *et al.*, 1996). This may also include microorganisms that are attached to particles, where the unattached surfaces of the microorganism are expected to be surrounded by pore water (de Blanc *et al.*, 1996). The top layers of the soil profile (soil zone) generally favour biodegradation due to an increased oxygen and nutrient content (Christensen and Larsen, 1993) and increased levels of oxygen at the uppermost regions of the soil profile have been shown to coincide with lower concentrations of organic contaminants (Davis *et al.*, 1992; Franzmann *et al.*, 2002). Although biological activity is the highest in the top layers of the vadose zone, microbial populations exist in significant numbers towards the water table (de Blanc *et al.*, 1996) and biodegradation within this region has

been reported (Stout and Lundegard, 1998). Soil type can also influence the amount of biodegradation, with soils containing high levels of oxygen and nutrients such as agricultural soil favouring biodegradation (Christensen and Larsen, 1993). However, biodegradation within leached sandy soils with low organic content also occurs (Davis *et al.*, 1998).

Areas of the vadose zone that are heavily saturated with petroleum NAPLs generally inhibit biodegradation due to several factors, such as the absence of sufficient oxygen and nutrients, the probable toxicity of high concentrations of petroleum components towards microbes, the low bioavailability of the petroleum and the lack of a water phase (Christensen and Larsen, 1993). As a result, biodegradation is favoured within areas of lower NAPL saturation (Stout and Lundegard, 1998), such as the fringe of a NAPL affected soil body (Christensen and Larsen, 1993) where hydrocarbon vapours distributed throughout the soil profile have been reported to undergo aerobic biodegradation (Davis *et al.*, 1998; Pasteris *et al.*, 2002; Johnson *et al.*, 2006), likely occurring in pore water and not the vapour phase (de Blanc *et al.*, 1996).

Anaerobic biodegradation occurs in the vadose zone after oxygen is depleted from pore waters by aerobic processes (de Blanc *et al.*, 1996). In sandy or gravelly vadose zones, oxygen can be introduced through water recharged from the surface. However, this introduced oxygen is often depleted in the vadose zone faster than it is replaced (de Blanc *et al.*, 1996). Infiltrating groundwater recharge may similarly introduce oxygen to the vadose zone, although it has been shown that oxygen can also be rapidly depleted in the groundwater (Salminen *et al.*, 2006). Anaerobic processes identified in the vadose zone include iron(III) reduction and methanogenesis. These processes have been identified as the major processes for the removal of petroleum hydrocarbons in light fuel, lubrication oil (Salminen *et al.*, 2006) and crude oil (Molins *et al.*, 2010) from contaminated vadose zones.

Groundwater

Petroleum biodegradation in groundwater is well documented (Barker *et al.*, 1987; Cozzarelli *et al.*, 1990; Borden, 1994; Cozzarelli *et al.*, 1995; Schmitt *et al.*, 1996; Hers *et al.*, 2000; Franzmann *et al.*, 2002; Tiehm and Schulze, 2003; Eriksson *et al.*, 2006). Components are generally first degraded by aerobic biodegradation processes where oxygen is present (Libelo *et al.*, 1998; Pinder and Celia, 2006). Aerobic microorganisms initially accumulate at the NAPL/water interface where petroleum components have the highest concentration in the water phase. However, components within the NAPL may possess significant toxicities towards microorganisms, resulting in biodegradation taking place within the water phase away from the NAPL/water interface (de Blanc *et al.*, 1996).

Anaerobic biodegradation typically occurs in groundwater after oxygen is depleted through aerobic processes, which is often rapid due to the low solubility of oxygen in water (Borden *et al.*, 1995; Libelo *et al.*, 1998; Burland and Edwards, 1999; Wiedemeier *et al.*, 1999). These processes occur within the dissolved contaminant plume where oxygen has been consumed.

Electron acceptors are generally utilised in a specific sequence according to the redox potential of the half-equations, and predictable redox zones are typically formed (i.e. nitrate reduction furthest from the source, followed by manganese (IV), iron (III) and sulfate reduction, and methanogenesis closest to the source (Fig. 1.2) (Franzmann *et al.*, 2002, Pinder and Celia, 2006).

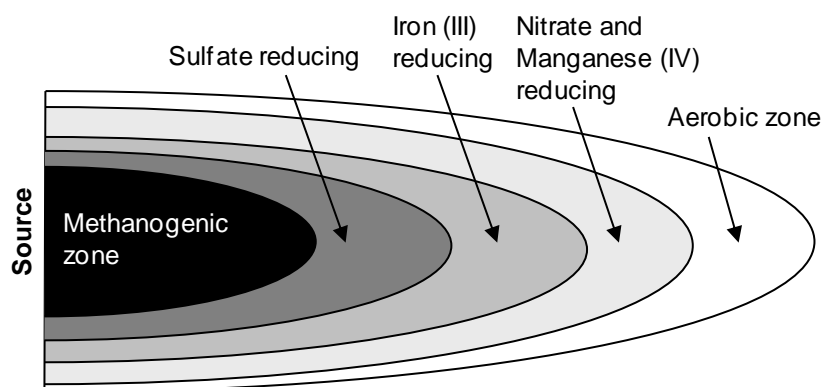


Figure 1.2 Redox zones within a plume of dissolved petroleum components. Adapted from Pinder and Celia (2006).

Petroleum biodegradation in the NAPL

Biodegradation of petroleum is generally considered to be limited by partitioning into the water phase (ITRC, 2009) as it is assumed that bacteria only biodegrade dissolved components (Cameotra and Makkar, 2010). However, aliphatic hydrocarbons are significantly affected by biodegradation processes and many of these compounds have negligible solubilities in water (Christensen and Larsen, 1993, Kaplan *et al.*, 1996). Several mechanisms have been proposed for the biodegradation of hydrophobic components by microorganisms. This includes the direct attachment of a microbial cell to a NAPL, and the production of biosurfactants (Rosenberg *et al.*, 1992; Fritsche and Hofrichter, 1999; ITRC, 2009).

The production of biosurfactants by hydrocarbon utilising bacteria under aerobic and anaerobic conditions has been well documented (Javaheri *et al.*, 1985; Hommel, 1990; Leahy and Colwell, 1990; Neu, 1996; Desai and Banat, 1997; Bredholt *et al.*, 1998; Bognolo, 1999; Ron and Rosenberg, 2002; Bodour *et al.*, 2003; Cameotra and Singh, 2009; Ganesh and Lin, 2009). Biosurfactants consist of molecules with hydrophilic and lipophilic moieties that act as emulsifiers, forming micelles that consequently lower the surface tension of a NAPL. These micelles accumulate at liquid/liquid, liquid/gas and

liquid/solid interfaces, allowing enhanced uptake and bioavailability of hydrophobic NAPL components in the water phase (Hommel, 1990; Fritsche and Hofrichter, 1999). Indigenous microbial populations isolated from petroleum contaminated subsurface sites have been shown to form biosurfactants in laboratory experiments (Bodour *et al.*, 2003; Bento *et al.*, 2005; Plaza *et al.*, 2006; Cameotra and Singh, 2009).

1.2.4.2 Compositional changes from biodegradation

Biodegradation processes can result in significant changes to the composition of petroleum after its release with the compound types altered in a specific order (Christensen and Larsen, 1993; Peters and Moldowan 1993; Kaplan *et al.*, 1996). Studies by Volkman *et al.* (1984), Peters and Moldowan (1993) and Kaplan *et al.* (1995) have described the apparent stepwise depletion of compounds in a specific order, based on their susceptibilities to biodegradation (possibly due to the compounds undergoing biodegradation at different rates). Table 1.1 shows the table presented by Kaplan *et al.* (1995), formed by comparison of unweathered petroleum (freshly dispensed gasoline, diesel and bunker C fuel) with the corresponding weathered petroleum (recovered from spill events). The type of biodegradation (aerobic or anaerobic) was not specified.

Table 1.1 Compositional changes to petroleum fuels with increased levels of biodegradation. Adapted from Kaplan et al. (1996).

Fuel type	Level of biodegradation	Chemical composition	
	1	Prominent <i>n</i> -alkanes	Increasing biodegradation ↓
	2	Lower molecular weight <i>n</i> -alkanes removed	
	3	Middle range <i>n</i> -alkanes, alkenes, benzene and toluene removed	
	4	Over 90% of the <i>n</i> -alkanes are removed	
	5	Alkylcyclohexanes and alkylbenzenes removed. Isoprenoids and naphthalene reduced	
	6	Isoprenoids, methylnaphthalenes, benzothiophene and alkylbenzothiophenes removed. C2-alkylnaphthalenes selectively reduced	
	7	Phenanthrenes, dibenzothiophene and other polynuclear aromatic hydrocarbons reduced	
	8	Tricyclic terpanes enriched. Regular steranes selectively removed. C33-C35-homohopanes reduced	
	9	Tricyclic terpanes, diasteranes and aromatic steranes abundant	
	10	Aromatic steranes abundant and demethylated hopanes predominant	

Aliphatic hydrocarbons

The relative resistances of aliphatic hydrocarbons to biodegradation correspond with increased carbon skeleton complexity. For example, *n*-alkanes have the simplest carbon skeletons in petroleum and are the most susceptible aliphatic compound type to biodegradation (Table 1.1), whereas branched and cyclic alkanes of greater carbon skeleton complexity possess a greater resistance (Kaplan *et al.*, 1996). This enhanced resistance shown by branched alkanes is typically observed in profiles of biodegraded petroleum products such as diesel, with a decrease of *n*-alkanes relative to the isoprenoids pristane and phytane (Atlas, 1981, Kaplan *et al.*, 1996). Low

molecular weight constituents of these aliphatic hydrocarbons are most susceptible to biodegradation, with their resistance to biodegradation generally higher with increasing molecular weight (Kaplan *et al.*, 1996). However, an opposite trend has been shown where biodegradation occurs as a result of methanogenesis, with higher molecular weight *n*-alkanes and cycloalkanes initially altered (Hostettler *et al.*, 2007).

The relative amounts of specific aliphatic hydrocarbons are often used to assess biodegradation levels of petroleum contaminants in the environment. Ratios are constructed using compounds that have similar physical properties (water solubilities and volatilities) so that changes to the ratios are only a result of biodegradation and not physical processes. Examples include *n*-heptadecane/pristane and *n*-octadecane/phytane, with decreased values relative to fresh petroleum indicating biodegradation (Kaplan *et al.*, 1996). Christensen and Larsen (1993) established a linear relationship that could be used to estimate the release date of a diesel fuel, using *n*-heptadecane/pristane ratios, where it was shown that the *n*-alkane *n*-heptadecane was completely removed after approximately 20 years (Christensen and Larsen, 1993). However, this rate may vary as a result of the subsurface conditions (Galperin and Kaplan, 2008).

Aromatic hydrocarbons

Aromatic hydrocarbons are also susceptible to biodegradation, which generally becomes evident after most of the *n*-alkanes have been removed (Fisher *et al.*, 1996; Kaplan *et al.*, 1996; Fisher *et al.*, 1998). The biodegradation of some aromatic compounds prior to *n*-alkanes of similar molecular weight has been reported, and suggested to be a result of their higher water solubilities and subsequent bioavailability (Fedorak and Westlake, 1981; Mao *et al.*, 2009). Aromatic hydrocarbon susceptibility to biodegradation decreases with increased molecular weight and carbon skeleton complexity (Fedorak and Westlake, 1981). For example, increased alkyl group length (Volkman *et al.*, 1984) and increased number of aromatic rings corresponds with a higher resistance to biodegradation (Kaplan *et al.*, 1996).

Aromatic compounds from petroleum can be used to assess biodegradation levels of petroleum contaminants. These biodegradation levels are assessed by observing changes to isomer distributions within aromatic compound groups, as specific isomers possess different susceptibilities to biodegradation (Fisher *et al.*, 1996; Fisher *et al.*, 1998; Trolie *et al.*, 1999; van Aarssen *et al.*, 1999). For example, it has been demonstrated that 1,2,3-trimethylbenzene is the least affected by moderate biodegradation compared with the remaining trimethylbenzene isomers (George *et al.*, 2002). Fisher *et al.* (1996) established the relative susceptibilities of di-, tri- and tetramethylnaphthalenes to biodegradation, where it was noted that isomers with 1,6-configuration were the most readily depleted isomers through biodegradation.

Unresolved complex mixtures

Extensive biodegradation of petroleum often results in the removal of resolved components in gas chromatograms and the relative enhancement of a “hump” (Blumer *et al.*, 1973; Frysinger *et al.*, 2003). This hump is usually referred to as an unresolved complex mixture (UCM), consisting of possibly thousands of individual compounds (Blumer *et al.*, 1973) that are generally considered recalcitrant (Gough and Rowland, 1990; Frysinger *et al.*, 2003). Dominant UCMs are commonly found in the analysis of in-reservoir biodegraded crude oils and petroleum contaminated environmental samples (Melbye *et al.*, 2009). Identification of compounds within a UCM using gas chromatography mass spectrometry (GC-MS) is often difficult due to interfering mass spectra of many different compounds. Studies characterising UCMs from petroleum indicate the presence of several compound types, usually compounds with relatively complex carbon skeletons. These include T-branched alkanes (Gough and Rowland, 1990), cycloalkanes (Killops and Aljuboory, 1990), monoaromatic compounds containing straight and branched chain alkyl groups (Warton *et al.*, 2000), and alkylindanes and alkyltetralins (Warton *et al.*, 2000). It has also been suggested that an increase in the relative abundance of polar compounds within petroleum as a result of weathering leads to an increased UCM (Lundegard and Sweeney, 2004).

1.3 Remediation

Remediation is the process whereby contamination is reduced in the environment. This may involve intrinsic processes (natural attenuation), or processes enhanced via human intervention. Remediation strategies can involve the physical removal of the petroleum or rely on weathering processes to reduce the contamination (Hoeppel *et al.*, 1991; Borden and Kao, 1992; Douglas *et al.*, 1992; Pearson and Oudijk, 1993; U.S.EPA, 1999; Salanitro, 2001; Chaîneau *et al.*, 2003).

1.3.1 Natural attenuation

Natural attenuation is an intrinsic remediation strategy that relies on naturally occurring weathering processes to reduce the concentration, mass, and toxicity of a released contaminant (Libelo *et al.*, 1998; Bolliger *et al.*, 1999; USEPA, 1999). Within the subsurface, non-destructive processes such as dissolution and volatilisation often result in reduced concentrations of contaminants, whereas destructive processes such as biodegradation often have a greater impact on the overall mass reduction of a contaminant (Bolliger *et al.*, 1999; Wiedemeier *et al.*, 1999). Biodegradation without human intervention is referred to as intrinsic bioremediation (Borden *et al.*, 1995; Wiedemeier *et al.*, 1995; Bolliger *et al.*, 1999) and the use of this monitored natural attenuation technique for the removal of petroleum has been well documented within the vadose zone (Chapelle and Lovley, 1992; Newman and Barr, 1997; Kristensen *et al.*, 2010) and groundwater (Kao and Wang, 2000; Kao and Prosser, 2001; Bekins *et al.*, 2005; Farhadian *et al.*, 2008; Kao *et al.*, 2010).

1.3.2 Physical and Chemical remediation techniques

Large volume petroleum contamination can be reduced in magnitude by recovering the free product. In the subsurface, these techniques involve the use of skimmer wells, trenches and pump wells (Charbeneau *et al.*, 2000).

Contaminated groundwater may also be extracted and treated through a variety of ex-situ processes. This method is known as pump and treat, and is one of the most widely used remediation techniques for contaminated groundwater (Damera and Bhandari, 2007). Soil may also be treated ex-situ through the excavation of the soil and its placement into heaps known as biopiles (Friend, 1996).

Soil vapour extraction or soil venting, is a common in-situ remediation technique that removes volatile and semi-volatile contaminants from the vadose zone (Thornton and Wootan, 1982; Johnson *et al.*, 1990; Annable *et al.*, 1993; Joo *et al.*, 1998). The technique utilises a pressure gradient formed through a vacuum, with contaminant vapours subsequently removed through extraction wells (Johnson *et al.*, 1990; Damera and Bhandari, 2007). Soil vapour extraction can be enhanced through steam injection and heating (Lingineni and Dhir, 1992; Joo *et al.*, 1998).

Air sparging is an in-situ remediation technique used to volatilise groundwater contaminants prior to their collection with soil vapour extraction techniques. Volatilisation is achieved through the injection of air into groundwater, carrying components upwards through the vadose zone for collection (Johnston *et al.*, 1998; Kirtland and Aelion, 2000; Benner *et al.*, 2002; Johnston *et al.*, 2002).

1.3.3 Bioremediation techniques

Bioremediation techniques facilitate the removal of petroleum contaminants by treatments that enhance biodegradation. These techniques can be applied in-situ and ex-situ (Korda *et al.*, 1997; Alvarez and Illman, 2006; Farhadian *et al.*, 2008).

In-situ biostimulation involves the addition of nutrients and electron acceptors (or donors) as amendments to promote biodegradation by indigenous microorganisms (Alvarez and Illman, 2006). Typical amendments for petroleum contaminated soils include fertilizers (Rosenberg *et al.*, 1992;

Walworth *et al.*, 1997; Duncan *et al.*, 2003; Sarkar *et al.*, 2005), molasses (Tsai *et al.*, 2009), compost, and biosolids (Namkoong *et al.*, 2002). Amendment addition may also be applied ex-situ in conjunction with biopiles (Friend, 1996). The addition of amendments to anoxic groundwater has been well documented, particularly in the biodegradation of monoaromatic hydrocarbons (reviewed by Farhadian *et al.* 2008). Studies show that the addition of sulfate, nitrate and iron(III) electron acceptors to petroleum contaminated anoxic aquifers often results in an increased rate and efficiency of anaerobic biodegradation for monoaromatic hydrocarbons (Cunningham *et al.*, 2001; Da Silva *et al.*, 2005; Sublette *et al.*, 2006).

Bioventing (Hoeppel *et al.*, 1991; Reisinger *et al.*, 1994; Vaneyk, 1994) and biosparging (Johnston *et al.*, 1998; Adams and Reddy, 2003) are two biostimulation techniques that are analogous to soil venting and air sparging respectively, and are applied in a similar manner. In addition to the previously mentioned physical remediation processes, these techniques also enhance aerobic biodegradation in the vadose and saturated zones through the injection of air and consequent introduction of oxygen (Hoeppel *et al.*, 1991).

Bioaugmentation involves the supplementation of indigenous, allochthonous or genetically modified microorganism species to assist biodegradation processes in soil and groundwater (Korda *et al.*, 1997; Mrozik and Piotrowska-Seget, 2010). Microorganisms can be selected based on their ability to degrade target compounds, as well as their ability to form biosurfactants (Francy *et al.*, 1991; Desai and Banat, 1997; Ron and Rosenberg, 2002; Cameotra and Makkar, 2010; Perelo, 2010).

1.4 Polar compounds formed from petroleum degradation

Petroleum degradation products can be formed through abiotic (Larson *et al.*, 1977; Burns, 1993) and biotic (Atlas, 1981; Gibson and Subramanian, 1984; Fedorak and Peakman, 1992; Heider *et al.*, 1998; Foght, 2008) processes. Degradation products are usually polar compounds with oxygen-containing

functional groups (in some instances multiple functional groups) (Atlas, 1981; Gibson and Subramanian, 1984; Wentzel *et al.*, 2007).

1.4.1 Abiotic degradation products

Abiotic degradation products of model compounds and petroleum are primarily documented as a result of photo-oxidation (Burwood and Spears, 1974; Larson *et al.*, 1977; Larson *et al.*, 1979; Payne and Phillips, 1985; Ehrhardt *et al.*, 1992; Ali *et al.*, 1995; Ehrhardt *et al.*, 1997; Nicodem *et al.*, 1997). Compound types formed include alcohols, ketones, aldehydes, carboxylic acids, fatty acids, esters, epoxides, sulfoxides, sulfones, phenols, anhydrides, quinones and hydroperoxides (Payne and Phillips, 1985; Lee, 2003).

Laboratory experiments have demonstrated the formation of a range of polar compounds from the simulated photo-oxidation of aliphatic (Guiliano *et al.*, 1997) aromatic (Ali *et al.*, 1995; Ehrhardt *et al.*, 1997; Moza *et al.*, 1999) and polar (heterocyclic) model compounds (Traulsen *et al.*, 1999). These studies have been conducted through partitioning experiments of NAPL and water, with the NAPL layer exposed to a light source (Burwood and Spears, 1974; Hansen, 1975). Analysis of the water phase dissolved organics by gas chromatography (GC) showed the presence of a UCM that increases in abundance throughout the experiments' duration (Burwood and Spears, 1974).

Photo-oxidation products identified in petroleum contaminated surface water environments and includes aldehyde, ketone, and alcohol derivatives of alkylbenzenes, indanes and fluorenes (Ehrhardt and Burns, 1990; Ehrhardt and Burns, 1993; Al-Lihaibi, 2003). The aqueous concentrations of these photo-oxidation products can be at least an order of magnitude greater than their parent hydrocarbons, which is likely due to their enhanced water solubility (Ehrhardt and Burns, 1993).

1.4.2 Biodegradation products

Established pathways of growth associated aerobic and anaerobic biodegradation show that a number of oxidised compounds form as intermediates prior to complete mineralisation to carbon dioxide and water (Atlas, 1981; Cerniglia, 1992; Heider *et al.*, 1998; Wentzel *et al.*, 2007; Foght, 2008). Co-metabolism on the other hand typically results in the formation of “dead-end” co-metabolites formed at the end of a biodegradation pathway, although potentially they can be further degraded by alternate microorganisms (Horvath, 1972; Foght, 2008).

1.4.2.1 Polar compounds from aerobic biodegradation

Aerobic biodegradation products can be formed from aliphatic and aromatic hydrocarbons (Atlas, 1981; Gibson and Subramanian, 1984; Cerniglia, 1992), as well as heterocyclic polar compounds (Kodama *et al.*, 1973; Mormile and Atlas, 1989; Fedorak and Peakman, 1992; Kropp *et al.*, 1994). Pathways commonly proceed through hydroxylation to form an alcohol, followed by further transformation to an aldehyde or ketone, and carboxylic acid where possible. Additional transformations can result in the formation of several intermediate compounds and end products that largely depend on the original structure of the compound metabolised (Gibson and Subramanian, 1984; Heider *et al.*, 1998; Fritsche and Hofrichter, 1999).

Aliphatic hydrocarbons

A range of aliphatic hydrocarbons form polar compounds through aerobic biodegradation. *n*-Alkanes generally undergo hydroxylation at the terminal position to form a primary alcohol, which is further oxidised to the corresponding aldehyde and carboxylic acid (Fig. 1.3) (Atlas, 1981; Wentzel *et al.*, 2007). Even numbered long-chain carboxylic acids (fatty acids) are further degraded through β -oxidation to the form, acetyl-coenzyme A (acetyl-CoA); a central intermediate that is completely oxidised or incorporated into biomass. Odd numbered carboxylic acids form propionyl-CoA, prior to further

degradation (Fritsche and Hofrichter, 1999). Subterminal oxidation of *n*-alkanes also occurs as a minor biodegradation pathway, resulting in the initial formation of secondary alcohols (Fig. 1.3) (Ashraf *et al.*, 1994; Whyte *et al.*, 1998). These can be further oxidised to the corresponding ketone and eventually transformed to an ester. Further degradation of the ester results in the formation of a primary alcohol and a carboxylic acid (Wentzel *et al.*, 2007). Branched alkane isoprenoids cannot undergo β -oxidation due to methyl group hindrance. As a result, isoprenoids form dicarboxylic acids through omega oxidation (Fig. 1.4) (McKenna and Kallio, 1971; Pirnik, 1977).

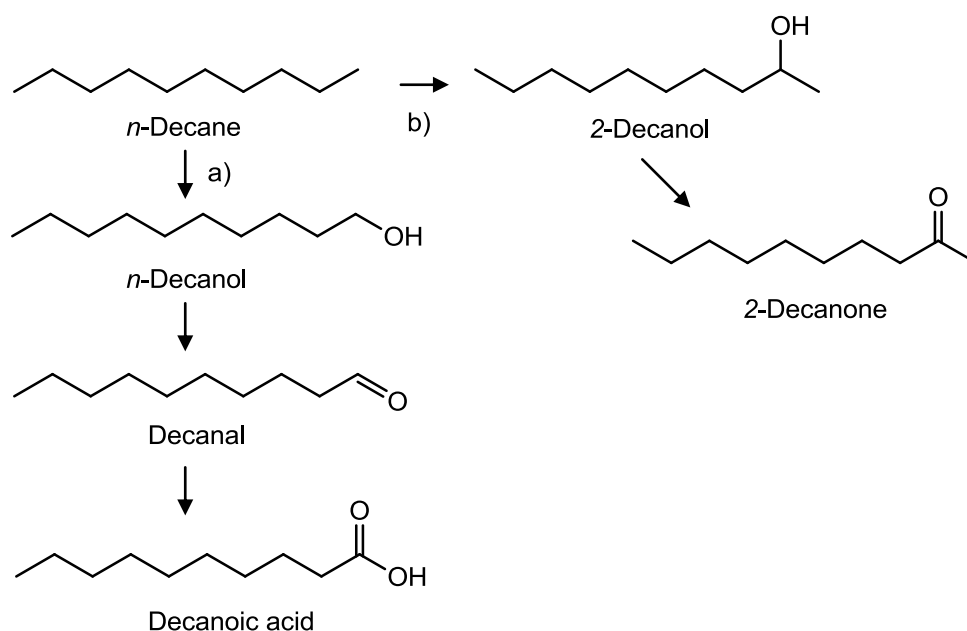


Figure 1.3 *n*-Alkane (*n*-decane) aerobic biodegradation pathway through a) terminal and b) subterminal oxidation.

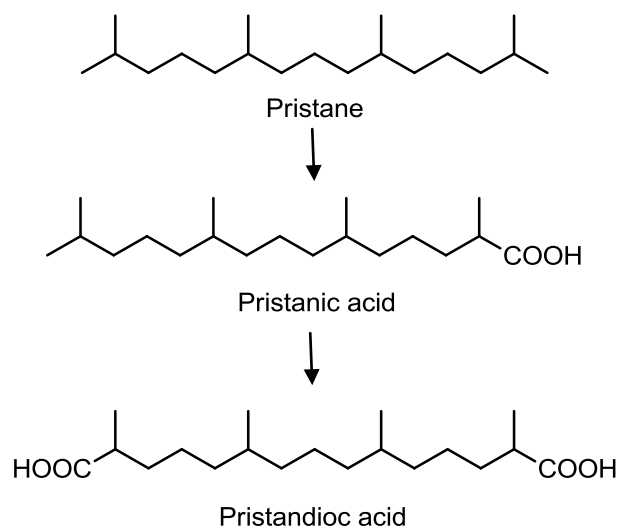


Figure 1.4 Pristane aerobic biodegradation pathway through omega oxidation. Adapted from McKenna and Kallio (1971).

Cycloalkanes and alkylcycloalkanes undergo oxidation primarily as a result of co-metabolic processes (Fritsche and Hofrichter, 1999). Unsubstituted cycloalkanes undergo oxidation forming alcohols and ketones (Atlas, 1981). Further transformation can occur, such as the formation of ϵ -caprolactone from cyclohexanone, prior to ring cleavage to form adipic acid and subsequent β -oxidation (Fig. 1.5) (Fritsche and Hofrichter, 1999). The presence of alkyl groups generally facilitates cycloalkane aerobic biodegradation, with alterations generally initiated upon the alkyl group, then following the general sequence described above (i.e. alcohol, aldehyde, carboxylic acid) (Atlas, 1981; Fritsche and Hofrichter, 1999).

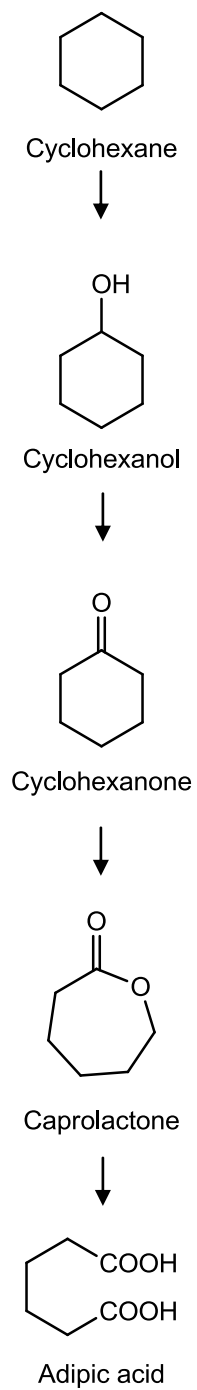


Figure 1.5 Cyclohexane aerobic biodegradation pathway. Adapted from Fritsche and Hofrichter (1999).

Aromatic hydrocarbons

A range of aromatic compounds undergo aerobic biodegradation to form polar compounds. These compounds undergo hydroxylation directly upon the aromatic ring, as well as upon alkyl groups present (Atlas, 1981; Cerniglia, 1992).

The aerobic biodegradation of benzene occurs through hydroxylation and dehydrogenation to form catechol, which is a common central intermediate for many aromatic compounds (Fig. 1.6) (Fritsche and Hofrichter, 1999). Further degradation of catechol occurs through two different ring cleavage pathways, resulting in the formation of several different intermediate metabolites (e.g. cis,cis-muconic acid (*ortho*- cleavage - Fig. 1.6a) and α -hydroxymuconic semialdehyde (*meta*- cleavage - Fig. 1.6b) (Gibson and Subramanian, 1984).

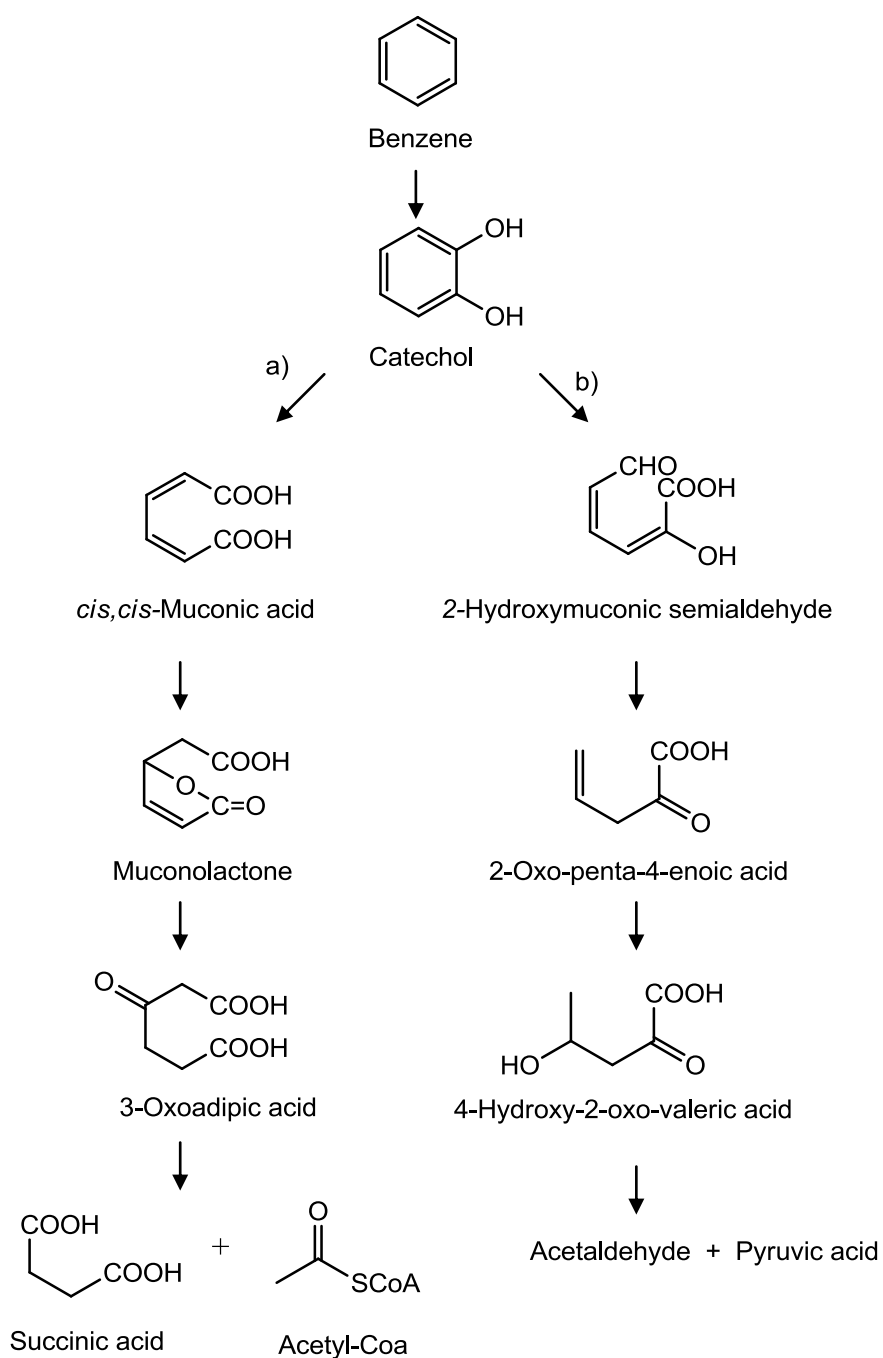


Figure 1.6 Benzene aerobic biodegradation pathway via the formation of catechol and subsequent *ortho*-cleavage to form a) *cis,cis*-muconic acid and b) *meta*-cleavage to form α -hydroxymuconic semialdehyde. Adapted from Fritsche and Hofrichter (1999).

Methylated monoaromatic compounds undergo hydroxylation to the aromatic ring (toluene can undergo hydroxylation at *ortho*-, *meta*- and *para*- positions) as well the methyl group (Gibson *et al.*, 1970; Shields *et al.*, 1989; Whited

and Gibson, 1991; Fritsche and Hofrichter, 1999). Ring hydroxylation can result in the incorporation of both atoms of molecular oxygen into the aromatic nucleus forming dihydroxylated intermediates, such as 3- and 4-methylcatechol from toluene (Shen *et al.*, 2000). Hydroxylation of monoaromatic compounds at the methyl group is followed by the transformations described for aliphatic hydrocarbons, forming aldehydes and carboxylic acids. For example, toluene hydroxylated at the methyl group initially forms benzaldehyde followed by benzoic acid (Shen *et al.*, 2000; Jindrova *et al.*, 2002), which is eventually transformed via the catechol intermediate (Fritsche and Hofrichter, 1999). Alkylated aromatic compounds undergo β -oxidation of the alkyl group resulting in the removal of two carbon atoms at a time. As a result, aromatic compounds with odd numbered alkyl groups are transformed to benzoic acid prior to transformation to catechol, whereas aromatic compounds with even numbered alkyl groups are transformed to phenylacetic acid (Gibson *et al.*, 1970).

Compounds containing aromatic and cyclic rings undergo aerobic degradation through oxidation of the cyclic or aromatic ring (Schreiber and Winkler, 1983; Sikkema and Debont, 1991). For example, tetralin forms 1-tetralol and 1-tetralone from oxidation of the cyclic ring (Schreiber and Winkler, 1983) as well as 5,6,7,8-tetrahydro-1-naphthalenol and 5,6,7,8-tetrahydro-2-naphthalenol from the oxidation of the aromatic ring (Sikkema and Debont, 1991).

Polycyclic aromatic hydrocarbons (PAHs) form polar compounds as products of aerobic biodegradation (Cerniglia, 1992). Established pathways commonly proceed via hydroxylation, with a range of polar compound types potentially formed (including alcohols, ketones and polar compounds with more than one functional group). For example, the aerobic biodegradation of naphthalene by fungi has been shown to produce 1-naphthol, 2-naphthol, *trans*-naphthalene-1,2-dihydrodiol, 4-hydroxy-1-tetralone, 1,2-naphthoquinone and 1,4-naphthoquinone (Cerniglia, 1992). Higher molecular weight PAHs such as fluoranthene and pyrene have been shown to form polar

biodegradation products prior to complete mineralisation, as well as dead-end products formed through co-metabolic processes (Cerniglia, 1992).

Heterocyclic compounds

Sulfur and nitrogen containing heterocyclic compounds undergo aerobic biodegradation to form a range of polar compounds (Gieg *et al.*, 1996; Xu *et al.*, 2006). Transformations can be initiated by attack upon carbon atoms or the heteroatom (Xu *et al.*, 2006).

Aerobic biodegradation pathways of heterocyclic sulfur compounds (e.g. benzothiophenes and dibenzothiophenes) are primarily co-metabolic (Fedorak and Grbicgalic, 1991; Saftic *et al.*, 1992; Kropp *et al.*, 1997b; Seymour *et al.*, 1997). Benzothiophene, dibenzothiophene and their methylated homologs undergo oxidation of the thiophene ring to form diones, as well as oxidation of the sulfur atom to form sulfones and sulfoxides (Kodama *et al.*, 1973; Mormile and Atlas, 1989; Fedorak and Grbicgalic, 1991; Saftic *et al.*, 1992; Kropp *et al.*, 1997a; Seymour *et al.*, 1997). An established aerobic biodegradation pathway for dibenzothiophene (Kodama pathway) proceeds through hydroxylation, followed by ring cleavage and formation of 3-hydroxy-2-formylbenzothiophene (Fig. 1.7a) (Kodama *et al.*, 1973; Kropp *et al.*, 1997a). This proposed pathway is also followed in the co-metabolism of dimethyldibenzothiophenes (Kropp *et al.*, 1997b). 2-Hydroxybiphenyl can be formed from the oxidation and subsequent removal of the sulfur atom (Fig. 1.7b) (Xu *et al.*, 2006).

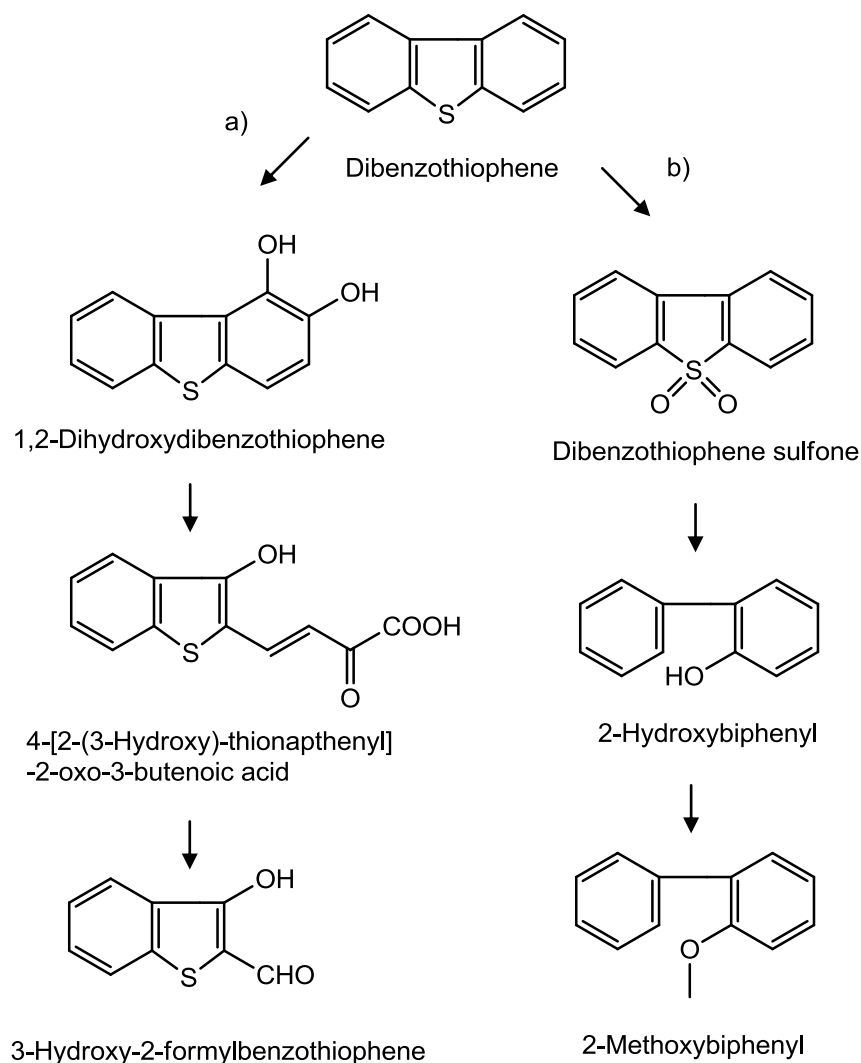


Figure 1.7 Dibenzothiophene aerobic biodegradation through the a) Kodama pathway and b) removal of sulfur heteroatom. Adapted from Xu et al. (2006).

Aerobic biodegradation of the heterocyclic nitrogen compound carbazole results in the formation of several degradation products with oxygen-containing functional groups (Gieg *et al.*, 1996; Xu *et al.*, 2006). This includes anthranilic acid formed via ring cleavage, which is further transformed to catechol (Gieg *et al.*, 1996). Carbazole can also undergo hydroxylation to form 3-hydroxycarbazole as a major metabolite, and 1- and 2-hydroxycarbazole as minor products (Lobastova *et al.*, 2004).

1.4.2.2 Polar compounds from anaerobic biodegradation

Anaerobic biodegradation products can be formed from aliphatic (So and Young, 1999; Wentzel *et al.*, 2007) and aromatic hydrocarbons (Leuthner and Heider, 2000; Spormann and Widdel, 2000; Annweiler *et al.*, 2002; Boll *et al.*, 2002; Foght, 2008), as well as heterocyclic polar compounds (Annweiler *et al.*, 2001; Safinowski *et al.*, 2006). Proposed pathways for anaerobic biodegradation are initiated by two reactions, namely, carboxylation (Zhang and Young, 1997; Spormann and Widdel, 2000; So *et al.*, 2003; Wilkes *et al.*, 2003; Callaghan *et al.*, 2006) and addition to the double bond of fumarate (Biegert *et al.*, 1996; Heider *et al.*, 1998; Kropp *et al.*, 2000; Boll *et al.*, 2002; Rios-Hernandez *et al.*, 2003; Meckenstock *et al.*, 2004; Callaghan *et al.*, 2006; Safinowski *et al.*, 2006; Foght, 2008).

Aliphatic Hydrocarbons

A range of aliphatic hydrocarbons can undergo anaerobic biodegradation to form biodegradation products. *n*-Alkanes undergo carboxylation, with the position of attack varied between different strains of bacteria (Wentzel *et al.*, 2007). For example, sulfate-reducing bacteria have been shown to carboxylate *n*-hexadecane at the C-3 position (So *et al.*, 2003), whereas an alternative strain carboxylated *n*-hexadecane at the C-2, C-4, and C-6 positions (So and Young, 1999). The addition of an *n*-alkane to the double bond of fumarate results in the formation of alkyl chain substituted succinates (Kropp *et al.*, 2000; Davidova *et al.*, 2005; Wentzel *et al.*, 2007).

Cycloalkanes also undergo carboxylation, and addition to fumarate to form succinates (Rios-Hernandez *et al.*, 2003; Wilkes *et al.*, 2003).

Aromatic hydrocarbons

A range of aromatic hydrocarbons form biodegradation products through anaerobic pathways. These pathways are initiated by a few reactions, including carboxylation (Zhang and Young, 1997), addition of the compound to fumarate (Widdel and Rabus, 2001; Safinowski *et al.*, 2006) and hydroxylation of alkyl groups (Spormann and Widdel, 2000) (Fig. 1.8). Mechanisms proposed for the anaerobic degradation of benzene include

carboxylation to form benzoic acid, methylation and addition to fumarate to form benzylsuccinic acid, and hydroxylation to form phenol. Phenol formed via hydroxylation can be further transformed via carboxylation to form *p*-hydroxybenzoic acid, or reduction to form cyclohexanone. Further degradation for all three pathways occurs through the central intermediate benzoyl-CoA (Foght, 2008).

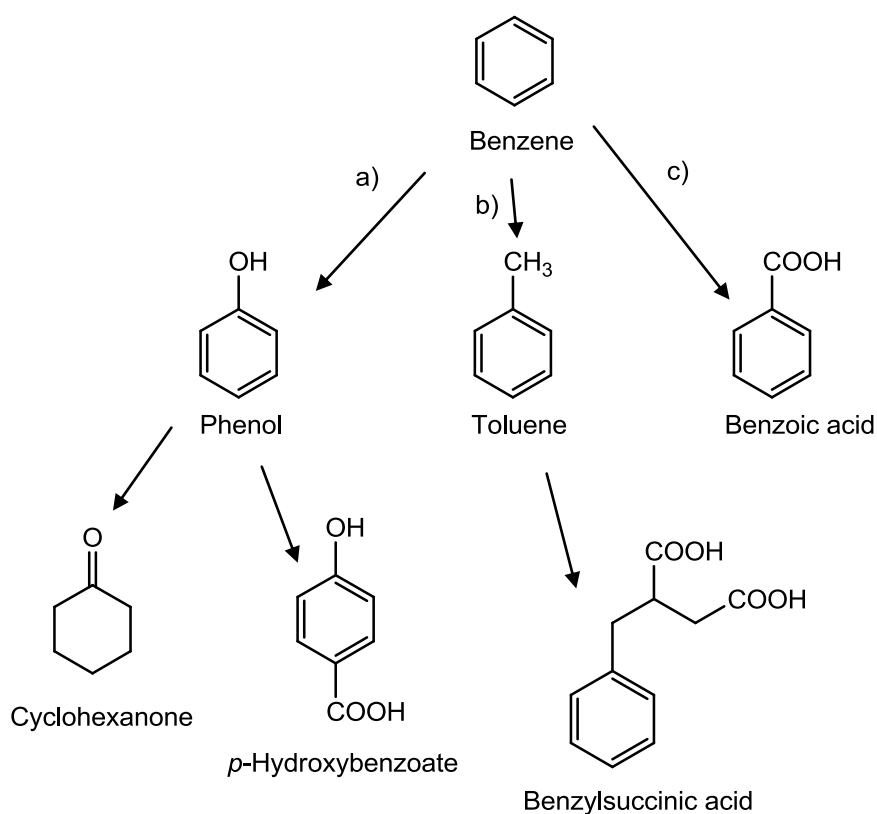


Figure 1.8 Proposed benzene anaerobic biodegradation pathways involving a) hydroxylation and subsequent ring reduction or carboxylation, b) methylation and subsequent addition to fumarate, and c) carboxylation. Adapted from Foght (2008).

Alkylbenzene anaerobic biodegradation also proceeds through the addition to fumarate to form the corresponding benzylsuccinic acid (Biegert *et al.*, 1996; Heider *et al.*, 1998; Leuthner and Heider, 2000; Spormann and Widdel, 2000; Boll *et al.*, 2002). For example, toluene, *p*-xylene, and ethylbenzene form benzylsuccinic acid, 4-methylbenzylsuccinic acid, and benzylethylsuccinic

acid respectively as anaerobic biodegradation products (Morasch and Meckenstock, 2005; Foght, 2008). *o*-Xylene has been shown to form 2-methylbenzylsuccinic acid as a co-metabolite of anaerobic biodegradation (Beller and Spormann, 1997).

Aromatic compounds with alkyl groups longer than one carbon in length undergo alternate anaerobic biodegradation pathways to carboxylation and the addition to fumarate (Heider *et al.*, 1998; Eriksson *et al.*, 2005). Ethylbenzene undergoes oxidation to form 1-phenylethanol prior to further transformation to acetophenone, and subsequent degradation through benzyl-CoA (Spormann and Widdel, 2000; Kniemeyer and Heider, 2001). The anaerobic biodegradation of propylbenzene can result in the formation of propylphenols (Eriksson *et al.*, 2005).

Anaerobic biodegradation pathways of naphthalene and alkylnaphthalenes can result in the formation of a range of biodegradation products (Fig. 1.9a). Initial reactions are similar to those suggested for the benzenes, and include carboxylation (Zhang and Young, 1997) and the addition to fumarate (Safinowski *et al.*, 2006). Naphthalene has been proposed to initially undergo methylation prior to the addition to fumarate, forming 2-(2-naphthalenylmethyl)succinic acid, which is further transformed to 2-methylnaphthoic acid (an intermediate that is analogous to the proposed naphthalene carboxylation pathway) (Zhang and Young, 1997; Safinowski *et al.*, 2006). Further transformation occurs via ring reduction and cleavage, resulting in the formation of several biodegradation products, including the dead-end metabolite decahydro-2-naphthoic acid (Fig. 1.9) (Annweiler *et al.*, 2002; Meckenstock *et al.*, 2004). Anaerobic biodegradation of 2-methylnaphthalene can follow the pathway proposed for naphthalene (2-methylnaphthalene is an intermediate of naphthalene anaerobic biodegradation) (Fig. 1.9b). 1-Methylnaphthalene can undergo co-metabolic methylation and subsequent addition to fumarate at the 2-position, which is followed by transformation to a dead-end product 1-methyl-2-naphthoic acid (Safinowski *et al.*, 2006).

Tetralin anaerobic biodegradation pathways include carboxylation of the aromatic ring to form 5,6,7,8-tetrahydro-2-naphthoic acid, which is an intermediate biodegradation product of naphthalene and 2-methylnaphthalene. Further transformations of tetralin follow those proposed for naphthalene and 2-methylnaphthalene (Fig. 1.9c) (Annweiler *et al.*, 2002; Meckenstock *et al.*, 2004).

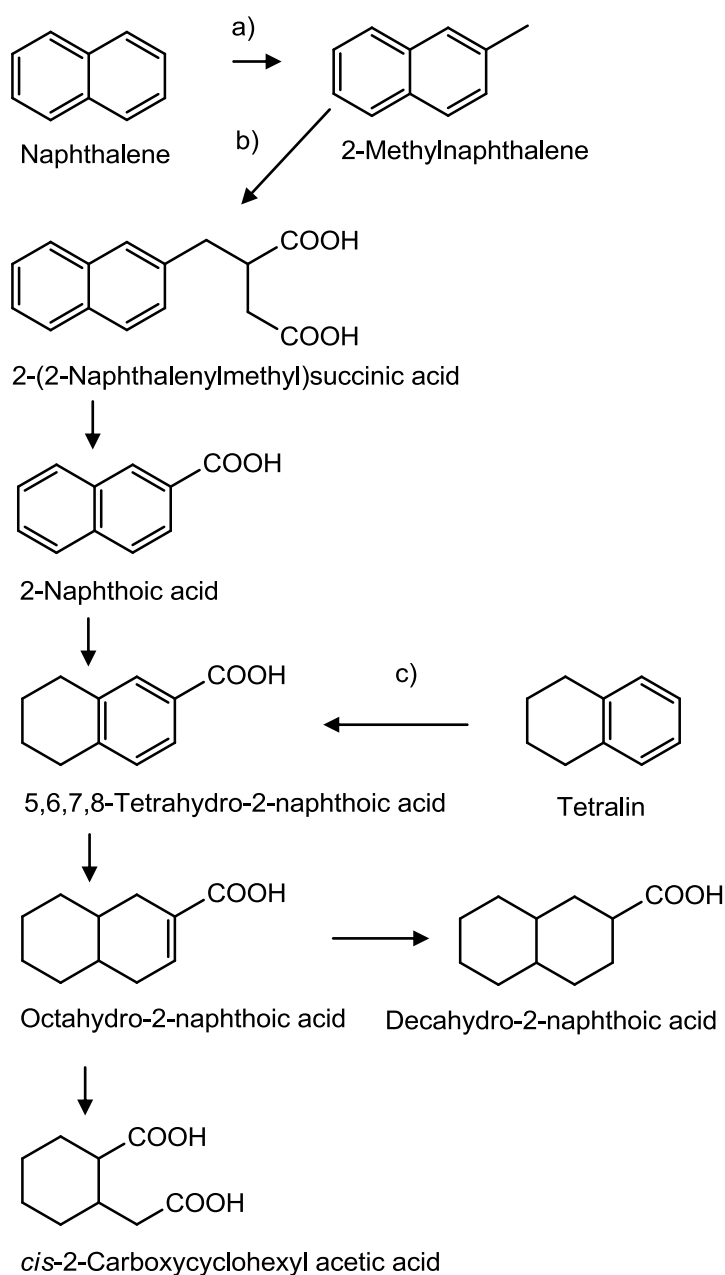


Figure 1.9 Proposed anaerobic biodegradation pathways for a) naphthalene, b) 2-methylnaphthalene and (c) tetralin. Adapted from Annweiler *et al.* (2002) and Foght (2008).

The anaerobic biodegradation of the additional aromatic compounds phenanthrene, and biphenyl can also occur, resulting in the formation of phenanthroic acid (Zhang and Young, 1997) and biphenyl-4-carboxylic acid (Selesi and Meckenstock, 2009) respectively.

Heterocyclic compounds

Heterocyclic aromatic compounds such as benzothiophene and benzofuran undergo co-metabolic anaerobic biodegradation in the presence of naphthalene substrates (Annweiler *et al.*, 2001; Safinowski *et al.*, 2006). Benzothiophene undergoes fumarate addition to form succinates (i.e. benzothiophenemethylsuccinic acid and benzothiophenemethylenesuccinic acid), which are subsequently transformed to carboxylated products (2-carboxybenzothiophene and 5-carboxybenzothiophene) (Annweiler *et al.*, 2001; Safinowski *et al.*, 2006). The oxygen containing heterocyclic compound benzofuran forms similar co-metabolites to benzothiophene, which include benzofuranmethylsuccinic acid, benzofuranmethylenesuccinic acid and benzofuranoic acid (Safinowski *et al.*, 2006).

1.4.3 Polar compounds formed in the vadose zone

The formation of polar compounds in laboratory experiments simulating petroleum biodegradation in the vadose zone has been demonstrated (Langbehn and Steinhart, 1995; Chaineau *et al.*, 2005; Mao *et al.*, 2009). Compound types identified from soil spiked with diesel and lubricating oil include branched and cyclic carboxylic acids, and aromatic alcohols, ketones and carboxylic acids. The major carbon skeleton types of these compounds relate to the composition of the original petroleum, with lubricating oil forming a higher relative abundance of branched alkane biodegradation products, and the diesel forming a higher relative abundance of aromatic biodegradation products (e.g. 1-hydroxy-2-naphthoic acid and 9-fluorenone-1-carboxylic acid) (Langbehn and Steinhart, 1995). Mao *et al.* (2009) also conducted a study simulating the biodegradation of petroleum in the vadose zone, analysing water leached organics from soil microcosms spiked with diesel. Compound types identified included aldehydes, ketones, diones,

lactones, alcohols, phenols and carboxylic acids, as well as some high molecular weight oxygenated sulfur and nitrogen containing compounds (Mao *et al.*, 2009).

Biodegradation products have also been identified in field studies associated with petroleum contaminated soils (Langbehn and Steinhart, 1995), including bioremediated soils (Angehrn *et al.*, 1998). Langbehn and Steinhart (1995) identified a series of polar compounds in soils affected by unknown petroleum contaminants. Polar compounds were suggested to have formed from the biodegradation of petroleum, and included cyclic carboxylic acids, and aromatic ketones, alcohols and diketones. The authors also suggested that biodegradation products such as cyclic carboxylic acids may persist within contaminated soils for several years. Polar compounds identified in ex-situ bioremediated soils affected by various petroleum contaminants (largely diesel and heating oil) include long chain alcohols, aldehydes, ketones, and carboxylic acids (Angehrn *et al.*, 1998).

1.4.4 Polar compounds formed in groundwater

Carboxylic acids are predominant compound types that are identified as biodegradation products in groundwater, most commonly detected within anoxic regions (Cozzarelli *et al.*, 1990; Cozzarelli *et al.*, 1994; Cozzarelli *et al.*, 1995; Beller, 2000; Namocatcat *et al.*, 2003). Carboxylic acids identified include those containing aliphatic, aromatic (Cozzarelli *et al.*, 1990; Cozzarelli *et al.*, 1995; Gieg and Suflita, 2002; Martus and Puttmann, 2003), and heterocyclic moieties (Annweiler *et al.*, 2001). The combined concentrations of these carboxylic acids in groundwater are often significantly higher than their parent compounds due to their higher aqueous solubilities (Cozzarelli *et al.*, 1995; Namocatcat *et al.*, 2003).

Aliphatic carboxylic acids identified in groundwater include low molecular weight carboxylic acids formed from crude oil (Cozzarelli *et al.*, 1994), and alkylsuccinic acids formed from gas condensates and other various hydrocarbon mixtures (Gieg and Suflita, 2002).

Aromatic carboxylic acids identified in groundwater associated with petroleum releases include a range of benzoic, benzylsuccinic, naphthoic and tetrahydronaphthoic acids, associated with gasoline (Cozzarelli *et al.*, 1995), jet fuel (Namocatcat *et al.*, 2003), gas condensates (Gieg and Suflita, 2002) and diesel (Gieg and Suflita, 2002). Heterocyclic carboxylic acids identified in groundwater include carboxybenzothiophene isomers associated with tar-oil (Annweiler *et al.*, 2001). Non-volatile organic acids (NVOAs) have been detected in groundwater contaminated with crude oil, with their molecular weights ranging between approximately 360 and 410 Dalton ($C_{18} - C_{22}$) (Thorn and Aiken, 1998). Although individual compounds were not identified (the authors suggested the NVOAs were too numerous for identification through GC-MS), bulk parameters measured indicated that the NVOAs were formed from alkylaromatic, alkylnaphthenoaromatic, and sulfur containing compounds, as well as possible degradation products of resins and asphaltenes.

Succinic acid biodegradation products which have been shown to form from alkylbenzenes and alkylnaphthalenes are exclusive to anaerobic processes (Ledin *et al.*, 2005), have a greater specificity to their parent hydrocarbons, and have been shown to possess a greater resistance to further degradation than simple carboxylic acids (Beller, 2000). As a result, succinic acid products may serve as indicators of natural attenuation through anaerobic biodegradation (Beller *et al.*, 1995; Gieg *et al.*, 1996; Beller, 2000).

1.4.5 Accumulation of polar compounds in petroleum NAPLs

The accumulation of carboxylic acids from in-reservoir biodegradation of crude oils has been documented (Meredith *et al.*, 2000; Barth *et al.*, 2004), and demonstrated in laboratory biodegradation experiments (Watson *et al.*, 2002; Erstad *et al.*, 2009). Watson *et al.* (2002) provided evidence that carboxylic acids may also accumulate from the biodegradation of surface spilled crude oil. The authors showed that the aerobic biodegradation of crude oil resulted in the formation of carboxylic acids that initially coincide with the depletion of the *n*-alkanes, and these acids are further degraded at a rapid rate. In contrast, branched and cyclic carboxylic acids formed from

biodegradation were resistant to further degradation, appearing as part of the UCM in gas chromatograms.

1.5 Total petroleum hydrocarbons

Risk assessments of petroleum contaminants in the environment are based on guidelines and criteria set by various agencies, and involve analytical techniques that measure petroleum contaminants to assess the level of remediation required at the site (TPHCWG, 1998). These techniques are usually aimed at specific groups of petroleum hydrocarbons, such as benzene, toluene, ethylbenzene and xylenes (BTEX) and PAHs (TPHCWG, 1998; Peters *et al.*, 1999; Xie *et al.*, 1999). Other analytical techniques used in the assessment of petroleum contaminants involve the measurement of the combined concentration of petroleum contaminants quantifiable with the method used (Hutcheson *et al.*, 1996; TPHCWG, 1998; Peters *et al.*, 1999). The most common method for assessing petroleum as a bulk parameter is total petroleum hydrocarbons (TPH), which as stated by Xie *et al.* (1999) “has become an accepted regulatory benchmark used widely to evaluate the extent of petroleum product contamination”.

Although the term TPH infers that only petroleum hydrocarbons are measured this may not be the case, as polar compounds (non-hydrocarbons) can also be incorporated in the analysis (TPHCWG, 1998; Zemo and Foote, 2003; Lundegard and Sweeney, 2004; Lundegard and Johnson, 2006). The presence of these polar compounds in TPH can make TPH based risk assessments difficult due to their presence as (or contribution to) a UCM or “hump” when analysed using GC-methods (Lundegard and Knott, 2001; Zemo and Foote, 2003). It has been suggested that for regulatory purposes a silica gel cleanup (EPA, 1996) step that removes polar compounds from TPH should be included, as the regulatory limits set for TPH are based on fresh petroleum products that contain largely petroleum hydrocarbons (Zemo and Foote, 2003). Sources of these polar compounds in TPH measurements include polar compounds from petroleum that are originally present within crude oil (Lundegard and Knott, 2001; Lundegard and Sweeney, 2004;

Lundegard and Johnson, 2006), polar compounds formed from the degradation of petroleum (Zemo and Foote, 2003; Lundegard and Sweeney, 2004), and polar compounds from NOM (Girard and Edelman, 1994; Lundegard and Sweeney, 2004).

1.5.1 Polar compounds in TPH from petroleum

Polar compounds present in petroleum have been well documented (Tissot and Welte, 1984) though they are usually present in very low abundances in light-middle crude oil fractions (Kaplan *et al.*, 1997). Polar compound types in crude oils include sulfur- (thiols, sulfides, disulfides and thiophenes), nitrogen (pyridines, quinolines, carbazoles and indoles (Seifert, 1970) and oxygen containing compounds (phenols, carboxylic acids, ketones, fluorenones and dibenzofurans) (Tissot and Welte, 1984). Resins and asphaltenes are also common constituents of crude oils, and consist of high molecular weight complex structures of polyaromatic and alkyl groups containing nitrogen, sulfur and oxygen (Tissot and Welte, 1984).

There are few reports of the presence of petroleum derived polar compounds in TPH extracted from groundwater. Lundegard and Knott (2001) reported the presence of dominant complex mixtures of polar compounds (average polar content of 83%) in TPH from groundwater associated with crude oil contamination. The high solubility of the polar compounds resulted in high concentrations of TPH, exceeding 10 mg/L in samples close to the NAPL source. Lundegard and Sweeney (2004) demonstrated that polar compounds from fresh diesel can be imparted to water through laboratory equilibration experiments. TPH extracts of water-accommodated fractions consisted of primarily polar compounds (96 % based on the removal of polar compounds with silica gel), with some individual polar compounds identified as alkylphenols.

The toxicity of petroleum-derived polar compounds is largely unknown compared to petroleum hydrocarbons (Lundegard and Knott, 2001; Eisentraeger *et al.*, 2008). However, studies have shown that some polar

compounds from petroleum are toxic (Seymour *et al.*, 1997; Eisentraeger *et al.*, 2008). These include alkylphenols (Tollefsen *et al.*, 2008a; Tollefsen *et al.*, 2008b), methylbenzothiophenes, dibenzothiophene (Seymour *et al.*, 1997), benzofuran, dibenzofuran, 2-methyldibenzofuran, 2,3-dimethylbenzofuran and carbazole (Eisentraeger *et al.*, 2008).

1.5.2 Polar compounds in TPH from the degradation of petroleum

The incorporation of degradation products in groundwater TPH analyses has been suggested by Zemo and Foote (2003). This study showed the presence of polar compounds in TPH extracts of groundwater impacted by weathered middle to high-boiling products of crude oil, that were observed by comparison of gas chromatograms before and after silica gel cleanup (polar compounds were removed after silica gel cleanup). Polar compounds in these samples were described as being present largely as a UCM.

The toxicities of polar compounds formed from the abiotic and biotic degradation of petroleum constituents are relatively unknown compared to their parent hydrocarbons. Polar compounds formed from the abiotic process photo-oxidation have been shown to be toxic. For example, photo-oxidation of a biodegraded crude oil has been shown to increase bioavailability, with water soluble fractions inducing toxic effects in the halophilic crustacean, *Artemia* (Maki *et al.*, 2001). It has also been suggested that water soluble fractions of photo-oxidised crude oil may be resistant to further degradation due to its toxicity towards microbes (Larson *et al.*, 1977; Larson *et al.*, 1979).

Polar compounds formed through biodegradation may be toxic (Ramirez *et al.*, 1996; Shen, 1998; Shelton *et al.*, 1999; Shen *et al.*, 2000; Chaineau *et al.*, 2003; Arias *et al.*, 2008). These polar biodegradation products include specific metabolites, such as BTEX metabolites 3-methylcatechol, 4-methylcatechol, 4-hydroxy-3-methoxybenzoic acid and 4-hydroxybenzoic acid, which have demonstrated cytotoxic effects in Hela cells (Shen, 1998).

Artificial biodegradation of weathered crude oil has been shown to produce toxic or mutagenic metabolites (Middaugh *et al.*, 1996; Shelton *et al.*, 1999; Middaugh *et al.*, 2002). In these studies, crude oil biodegradation led to the accumulation of water soluble compounds that produced greater toxicities upon embryonic and larval inland silversides (*Menidia beryllina*) (Middaugh *et al.*, 1996), embryonic grass shrimp (*Palaemonetes pugio*) (Shelton *et al.*, 1999) and larval mysids (*Mysidopsis bahia*) (Shelton *et al.*, 1999) than non-biodegraded crude oil. However, it was not determined whether the increased toxicity is a result of the formation of toxic metabolites, or the formation of metabolites that may act as co-solvents for toxic crude oil compounds (Shelton *et al.*, 1999). Another study by Melbye *et al.* (2009) involving artificially weathered in-reservoir biodegraded crude oil found that the water soluble fractions of the crude oil induced toxic effects in rainbow trout. The authors suggest this toxicity was largely a result of a polar fraction that accounted for 70% of the water soluble fraction and was dominated by a UCM. Individual components identified in this fraction included of cyclic and aromatic sulfoxides, though it was not determined whether these compounds were formed from the weathering processes or original crude oil components (Melbye *et al.*, 2009). Another study also showed that the weathering of volatile crude oils can result to an increase in toxicity, which was found to correspond with the evaporative losses to the crude oils (Evans *et al.*, 2000).

Studies documenting the toxic effects produced by artificially biodegraded crude oil in laboratory experiments are backed by field studies involving the bioremediation of crude oil in soil, where the toxicity of soils amended with fertilizer has been shown to persist after hydrocarbon concentrations have been reduced. This was suggested to be a result of the metabolites formed (Chaineau *et al.*, 2003).

In contrast to the previously mentioned literature, microbial transformations of petroleum hydrocarbons and polar compounds have also been shown to reduce risk by producing compounds with lower toxicities (Sutherland, 1992; Seymour *et al.*, 1997). For example, PAH metabolism by fungi has been shown to form transformation products with generally lower toxicities than

their parent hydrocarbon (Sutherland, 1992). Similar results have been shown for alkylbenzothiophene and dibenzothiophene metabolites (including diones, sulfones and sulfoxides) with biodegradation products generally exhibiting lower toxicities than their parent thiophene. However, the same study showed that the biodegradation products were far more water soluble than their parent thiophene (and therefore capable of higher concentrations in water) and were still toxic (Seymour *et al.*, 1997).

1.5.2.1 Polar compounds from natural organic matter (NOM)

NOM is a broad term that is often used to describe ubiquitous naturally occurring organic compounds derived from plants, animals and microorganisms (Christy and Egeberg, 2000; Chen *et al.*, 2002; Guo *et al.*, 2003). NOM can be comprised of a number of biochemical precursors, such as carbohydrates (polysaccharides, monosaccharides), proteins, peptides, lipids, amino sugars, lignins and tannins (Thurman, 1985b; Gadel and Bruchet, 1987; Kordel *et al.*, 1997; Lehtonen *et al.*, 2001; Leenheer *et al.*, 2003; Otto and Simpson, 2007). NOM is often described in terms of two operationally defined fractions known as humic (hydrophobic) and non-humic (hydrophilic) substances (Davis and Gloor, 1981; Leenheer, 1981; Collins *et al.*, 1986; Marhaba and Pu, 2000).

Non-humic substances largely consist of unaltered primary biochemicals such as carbohydrates, amino acids and lipids. This fraction typically accounts for 20-30% of NOM in soil and 20 % of NOM in aquatic environments (Thurman, 1985b). Carbohydrates are organic molecules that contain only carbon, hydrogen and oxygen, with the simplest carbohydrates (monosaccharides) typically consisting of cyclic, polyhydroxylated ketones and aldehydes (Killops and Killops, 2005). Amino acids are molecules consisting of an amine and carboxylic acid group attached to a common carbon atom. The carbon atom belongs to a side chain that varies between different amino acid types that can include alkyl substituents containing carboxylic, phenyl, phenol and amide groups (Killops and Killops, 2005).

Lipids include a wide variety of compound classes such as glycerides, waxes and terpenoids. Simple molecules within lipids include long-chain carboxylic acids (fatty acids), and alcohols (fatty alcohols), and these simple molecules are commonly combined with other structures to make up different lipid types (Fig. 1.10). Terpenoids are a class of lipids that are constructed from isoprene units, which are further separated into classes based on the number of isoprene units present. For example, monoterpenoids (2 units), sesquiterpenoids (3 units), diterpenoids (4 units) sesterterpenoid (5 units), triterpenoids (6 units) and tetraterpenoids (8 units). The result of these configurations is a range of compounds containing carbon skeletons based on the isoprene rule, which can also contain a range of polar functionalities (Killops and Killops, 2005). Terpenoids can be related to specific sources as a result of their higher concentrations. This includes phytol present in chlorophylls (Ellsworth and Perkins, 1966) and abietic acid present in conifer resins (Fig. 1.10) (Wakeham *et al.*, 1980).

Non humic substances can also be derived from the microorganisms that biodegrade petroleum. This includes cellular substances such as fatty acids and triglycerides, which have been suggested to be incorporated into residual NAPL fractions (Riis *et al.*, 1996).

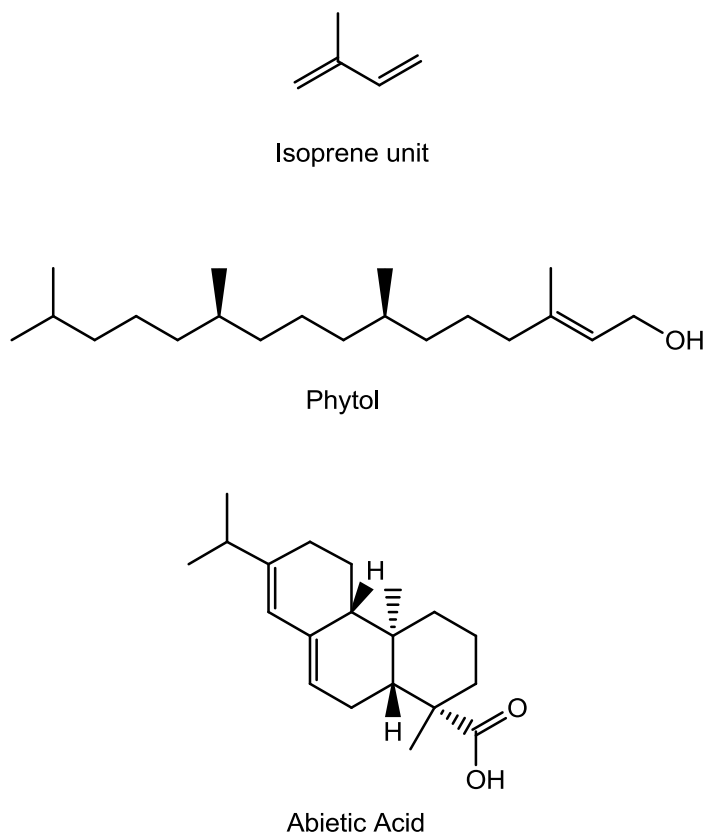


Figure 1.10 An isoprene unit, and phytol and abietic acid terpenoids.

Humic substances consist of a heterogeneous mixture of polyfunctional macromolecular polymers formed from complex humification processes (Aiken *et al.*, 1985; Stevenson, 1985). Humic substances have a large molecular weight range, from several hundred to greater than 300,000 Dalton (Stevenson, 1985). They typically account for 60-80 % of NOM in soils, 50-70 % of NOM in freshwaters and up to 90 % of NOM in highly coloured waters (Aiken, 1985). The percentage of humic substances in groundwater is often lower than surface water, with 50 % of organic solutes in groundwater consisting of hydrophilic acids (Thurman, 1985a; Malcolm, 1993).

Polar compounds from NOM have been observed in the TPH (or similar analyses) of soil (Wang *et al.*, 2009) and groundwater (Girard and Edelman, 1994) where silica gel cleanup methods were not implemented. However, these interferences are rarely documented. Girard and Edelman (1994)

conducted an investigation into TPH extracted from groundwater downgradient from a wood waste disposal site that was originally reported as diesel. TPH extracts of diesel range organics consisted of a large UCM that did not contain specific diesel components. Tannin and lignin derived materials in groundwater samples were measured using Standard Method 5550B, a colorimetric method for aromatic hydroxyl groups (Clesceri *et al.*, 1998). Results showed a direct correlation between diesel range TPH and tannin and lignin measurements with the colorimetric method. The authors suggested that the TPH was a result of semi-volatile degradation products of tannin and lignin, as these components would not be sufficiently volatile to be detected (Girard and Edelman, 1994). However, specific NOM components were not identified within TPH extracts.

Interferences from NOM in soil TPH are also rarely documented. It has been suggested that NOM from soil (referred to as biogenic organic compounds; BOCs) can be easily misidentified and quantified as petroleum hydrocarbons using comparable methods to TPH (Wang *et al.*, 2009). BOCs identified in storm water management ponds include polar compounds, such as sterols, sterones, fatty acids, fatty alcohols, waxes and wax esters (Wang *et al.*, 2009).

1.6 Characterisation techniques

A wide range of techniques have been previously used in the characterisation of NOM and polar compounds related to petroleum. For example, elemental analysis (Riis *et al.*, 1996; Thorn and Aiken, 1998; Croue *et al.*, 2000), Fourier transform infrared spectroscopy (FTIR) (Riis *et al.*, 1996; Croue *et al.*, 2000; Chen *et al.*, 2002), nuclear magnetic resonance (NMR) (Thorn and Aiken, 1998; Croue *et al.*, 2000; Cook, 2004), enhanced GC-techniques (Skoczynska *et al.*, 2008; Mao *et al.*, 2009; Melbye *et al.*, 2009), pyrolysis GC-MS (Greenwood *et al.*, 2006; Berwick, 2009), hydrogenation (Sandison *et al.*, 2003) and dehydrogenation (Huebner and Jacobs, 1947b; Suginome and Kakimoto, 1959; Kobayashi and Akiyoshi, 1962).

1.6.1 Elemental analysis

Elemental analysis has been used to analyse petroleum contaminated soil (Riis *et al.*, 1996) and groundwater (Thorn and Aiken, 1998), as well as the characterisation of NOM (Croue *et al.*, 2000). Target elements that are common to petroleum and NOM include carbon, hydrogen, oxygen, sulfur and nitrogen (Riis *et al.*, 1996; Thorn and Aiken, 1998; Croue *et al.*, 2000). Riis *et al.* (1996) used elemental analysis to show an increased oxygen content in petroleum as a result of biodegradation in laboratory experiments. Elemental analysis has also been used to provide evidence for petroleum derived organic matter in groundwater. For example, Thorn and Aiken (1998) showed that oxidation products of petroleum (NVOAs) in groundwater associated with crude oil contamination contained a higher sulfur content than naturally occurring NVOAs in uncontaminated groundwater. This was suggested to be a result of the sulfur containing NVOAs forming from sulfur-containing crude oil components.

1.6.2 Fourier transform infrared spectroscopy (FTIR)

FTIR has been used in the analysis of polar compounds in petroleum contaminated soil (Riis *et al.*, 1996), biodegraded crude oil (Barth *et al.*, 2004; Erstad *et al.*, 2009) and the characterisation of NOM (Croue *et al.*, 2000; Chen *et al.*, 2002). This technique can provide qualitative information of the major polar functional groups through their characteristic infrared absorption bands. Riis *et al.* (1996) identified polar functional groups from long term petroleum contaminated soils using FTIR. FTIR has also been used to characterise acidic compound types (i.e. aliphatic carboxylic acids and phenols) accumulated in biodegraded crude oil (Barth *et al.*, 2004; Erstad *et al.*, 2009).

The characterisation of NOM with FTIR provides strong absorption bands that relate to predominant features of isolated NOM (and therefore relate to specific NOM types). For example, acidic groups of humic substances, esters

of specific tannins or amides of proteins and amino sugars. FTIR also allows relative abundances of carbohydrate and hydrocarbon components to be determined (Croue *et al.*, 2000). However, the use of a bulk analysis technique like FTIR that can only identify functional group types present in a polar UCM may be difficult to apply to distinguishing NOM from petroleum derived polar compounds.

1.6.3 Nuclear magnetic resonance spectroscopy (NMR)

NMR has been used in the analysis of NVOAs from natural and crude oil contaminated groundwater (Thorn and Aiken, 1998) and in the characterisation of NOM (Croue *et al.*, 2000; Cook, 2004). Chemical shifts from NMR spectra give qualitative compound information, including polar functional groups. Thorn and Aiken (2008) used ^{13}C and ^1H NMR to show differences in NVOAs from natural and crude oil contaminated groundwater. Naturally occurring groundwater NVOAs possessed a higher carboxylic acid and heteroaliphatic content than those associated with crude oil contamination, which contained a higher aliphatic and methyl substituted aromatic proportion.

The characterisation of NOM with ^{13}C and ^1H NMR provides chemical shifts that relate to specific functionalities of isolated NOM. These include oxygen containing heteroaliphatic structures of carbohydrates, aliphatic and aromatic carbon, phenolics, ketones, aldehydes, carboxylic acids and amides (Malcolm, 1990). However, similar to FT-IR the use of a bulk analysis technique like NMR that can only identify functional group types present in a polar UCM maybe difficult to apply to distinguishing NOM and petroleum derived polar compounds.

1.6.4 Gas chromatography techniques

The presence of polar compounds as a UCM in environmental samples associated with petroleum contamination often limits identification of specific compounds. However, advancements in GC-techniques may enable satisfactory separation of components for identification. In addition, some chemical transformations may be applied to polar compounds present as a UCM prior to analysis and may produce resolvable components that can be identified using GC-MS.

Two-dimensional gas chromatography (GCxGC) is an enhanced GC-technique that has been used to analyse petroleum UCMs (Frysinger *et al.*, 2003; Van de Weghe *et al.*, 2006; Skoczynska *et al.*, 2008). The technique utilises two columns in succession that usually have different properties, separating the components through two different mechanisms (i.e. volatility and polarity) (Frysinger *et al.*, 2003). The major advantage of GCxGC over conventional one-dimensional GC is a higher peak capacity, resulting in an improved separation of the components (Skoczynska *et al.*, 2008; Cortes *et al.*, 2009). By coupling GCxGC with time of flight mass spectrometry (ToF-MS) at high acquisition rates, compound specific information can be obtained (Skoczynska *et al.*, 2008). GCxGC methods are useful in the rapid separation and identification of compound types (separated into groups based on their similar interaction with the different GC-phases) and the tentative identification of unknown compounds within UCMs (Beens *et al.*, 1998; Frysinger *et al.*, 2003; Van de Weghe *et al.*, 2006; Skoczynska *et al.*, 2008).

GCxGC techniques have been applied to petroleum UCMs from contaminated environments (Frysinger *et al.*, 2003; Nelson *et al.*, 2006; Van de Weghe *et al.*, 2006), with the majority of these studies identifying hydrocarbon components. GCxGC-ToF-MS has been used to identify polar compounds from petroleum contaminated sediments (Skoczynska *et al.*, 2008), polar fractions of artificially weathered, in-reservoir biodegraded crude oil (Melbye *et al.*, 2009) and water leached from diesel spiked soil microcosms (Mao *et al.*, 2009). GCxGC has been applied to petroleum

contaminated soil using a method comparable to TPH (Van de Weghe *et al.*, 2006). The authors suggested GCxGC techniques offered more specific compositional information than conventional TPH and eliminated the need for separation of aliphatic and aromatic compound types through additional sample preparation (silica gel fractionation) (Van de Weghe *et al.*, 2006; Seeley *et al.*, 2007).

A disadvantage of advanced GCxGC techniques is the added expense of additional instrumentation (particularly where TOF-MS is involved (Van de Weghe *et al.*, 2006), which is not typically utilised in the routine analysis of petroleum contaminated environmental samples (e.g. TPH). GCxGC also relies on the separation of the analytes in either column for the identification of a component, and the polar compounds present in TPH extracts associated with weathered petroleum may be too similar to be separated. This has been shown for UCMs from bitumens extracted from late Achaean sediments, where GCxGC was able to resolve some *n*-alkane components, whereas a UCM of cyclic components was left unresolved (cyclic compounds within the UCM contained similar fragmentation patterns, and the monitoring of specific ions could not resolve individual components) (Ventura *et al.*, 2008).

1.6.4.1 Pyrolysis GC-MS

Pyrolysis-GC-MS is widely used in the characterisation of NOM (Wershaw and Bohner, 1969; Saizjimenez *et al.*, 1978; Saizjimenez and Deleeuw, 1986; Gadel and Bruchet, 1987; Schulten, 1993; Hatcher and Clifford, 1994; Saizjimenez, 1994a; Christy *et al.*, 1999; Schulten and Gleixner, 1999). These techniques commonly utilise high temperatures that cleave macromolecular NOM to form GC-amenable fragments characteristic of specific origins (Christy *et al.*, 1999). A disadvantage of high temperature pyrolysis is the rearrangement of carbon skeletons through secondary reactions, such as isomerisation, alkylation and cyclisation (Saizjimenez, 1994a). These techniques are difficult to apply to TPH extracts containing

semi-volatile components that will volatilise in the pyrolysis chamber rather than pyrolyse, such as free fatty acids (Saizjimenez, 1994b).

Pyrolysis techniques can also be performed using off-line closed systems under milder conditions, such as micro-scale sealed vessel pyrolysis (MSSV) (Greenwood *et al.*, 2006; Berwick, 2009). MSSV has been utilised in the characterisation of bacterial biomarker compounds in natural waters, reducing the functionality of bacteriohopanepolyols by the removal of their polyhydroxylated side chain (Greenwood *et al.*, 2006). This technique has been shown to removal of polar functionalities, and the application of the technique to semi volatile polar compounds present as a UCM in TPH extracts may produce compounds (hydrocarbons) that could be used to distinguish NOM and petroleum-derived polar compounds.

1.6.4.2 Hydrogenation

Hydrogenation has been used to characterise polar UCMs from lignite and shale. Hydrogenation is a chemical reaction where organic compounds are reduced (saturated) through the addition of hydrogen atoms (Augustine, 1965; Rylander, 1979). For example, alkene and aromatic compounds hydrogenate to form the corresponding alkane and cycloalkane respectively. Hydrogenation can also involve the cleavage of carbon-carbon or carbon-heteroatom bonds. This is referred to as hydrogenolysis and can be applied to the defunctionalisation of polar compounds (Carruthers and Coldam, 2004). Catalytic hydrogenation often involves the mixing of an organic compound dissolved in solvent under a hydrogen atmosphere in the presence of a catalyst (e.g. platinum or palladium) (Augustine, 1965; Carruthers and Coldam, 2004). This process can also be carried out on-line in conjunction with GC techniques, by coupling a heated chemical reactor to the inlet or outlet of a GC column. Chemical reactors often contain a fused silica capillary internally coated with a platinum or palladium catalyst, using the hydrogen carrier gas for hydrogenation (Henneberg *et al.*, 1975; Schomburg *et al.*, 1982; Lequere *et al.*, 1989). On-line hydrogenation can also be carried out without a separate reactor, taking place within a

vaporising injector containing the catalyst (Sandison *et al.*, 2003). Through the application of this method to model compounds, Sandison *et al.* (2003) hydrogenated alkenes and aromatic compounds, desulfurised thiols, sulfides and thiophenes, and defunctionalised alcohols, acids, esters and ethers. Sandison *et al.* (2003) also applied the technique to polar fractions isolated from sediment extracts (lignite and shale) that originally showed a large UCM when analysed by GC-MS. Hydrogenation of these fractions produced a series of resolved hydrocarbon components, which included *n*-alkanes and triterpenoids. A disadvantage of this technique is the large amount of sample required, as polar fractions injected (1 µL) for on-line hydrogenation by Sandison *et al.* (2003) contained high concentrations (20 – 30 mg/mL).

1.6.4.3 Dehydrogenation

Dehydrogenation has been applied to aliphatic UCMs from petroleum (Farcasiu and Rubin, 1987; Gallacher *et al.*, 1991) and the elucidation of natural product carbon skeletons (Craig and Jacobs, 1944; Huebner and Jacobs, 1947a; Huebner and Jacobs, 1947b; Sugimoto and Kakimoto, 1959; Kobayashi and Akiyoshi, 1962). Dehydrogenation is a chemical reaction whereby hydrogen atoms are removed from organic compounds, often performed by heating the compound off-line in a sealed vessel that contains a catalyst (e.g. sulfur, selenium, platinum or palladium) (Fu and Harvey, 1978). Catalytic dehydrogenation has been used in dehydrocyclisation, aromatisation and defunctionalisation reactions (Fu and Harvey, 1978; Bastow *et al.*, 2001; Lang *et al.*, 2005).

The application of dehydrogenation to UCMs of petroleum has been shown to reduce the complexity of the UCM, through the aromatisation of aliphatic cyclic rings and consequent reduction in isomers present. Gallacher *et al.* (1991) used a palladium on carbon (Pd/C) catalyst to dehydrogenate naphthenic groups in a heavy crude oil distillate, reducing the complexity of the UCM so that ¹³C NMR could be used to elucidate naphthenic groups.

Natural product chemists elucidated the carbon skeleton of natural products by carrying out reactions that simplified the compound for identification (Kobayashi and Akiyoshi, 1962). These techniques included dehydrogenation reactions that defunctionalised and aromatised the natural products to form aromatic hydrocarbons with simpler configurations and fewer possible isomers. The identification of the basic ring system of a natural product was an invaluable step in the identification of natural products (Craig and Jacobs, 1944; Huebner and Jacobs, 1947a; Huebner and Jacobs, 1947b; Sugimoto and Kakimoto, 1959; Kobayashi and Akiyoshi, 1962).

1.7 Scope and objectives of this study

The research is aimed at characterising complex mixtures of polar compounds present in TPH associated with weathered petroleum. This includes investigations into the formation and accumulation of polar compounds associated with weathered petroleum, and the development of methodology to distinguish the source (petroleum or NOM) of complex mixtures of polar compounds present in TPH.

Chapter 2 investigates the occurrence of polar compounds present as a UCM in groundwater TPH downgradient from a weathered diesel source.

Compound class characterisation was carried out on the TPH extracts to assess the proportion of polar components present, and GC-MS techniques applied to fingerprint TPH samples, as well as assessing the extent of biodegradation with increasing distance from the source.

Chapter 3 investigates the accumulation and dissolution of polar compounds present as a UCM in weathered diesel. Compound class characterisation is used to assess the major compound classes present in fresh and weathered diesels and their dissolved water phases. Biodegradation of the weathered diesel was identified as the likely source of the polar compounds that have accumulated in the weathered diesel. The potential differences in the risk posed by fresh and weathered diesels to groundwater is discussed.

In Chapter 4, polar compounds (previously reported and unreported) present in polar UCMs associated with weathered petroleum from soil, equilibrated water, groundwater and NAPL samples were identified. The possible origins of these compounds, and insights into the location these polar compounds (and polar UCMs) form in the subsurface was assessed based on their presence or absence in the soil, equilibrated water and groundwater samples. The likely carbon skeleton types present in the polar UCMs were also assessed based on diagnostic ions present in mass spectra of the polar UCMs.

In Chapter 5, a novel technique for differentiating the source (petroleum or NOM) of polar UCMs present in TPH extracts was developed. The technique utilises off-line dehydrogenation to defunctionalise and aromatise polar UCMs to produce compounds that are easily identified using GC-MS, and can be related to NOM or petroleum based on their carbon skeletons. A series of model compounds were used to optimise reaction conditions prior to the technique's application to polar UCMs isolated from TPH extracts associated with NOM and weathered diesel.

In Chapter 6, a simple screening tool to differentiate the source (petroleum or NOM) of polar UCMs present in groundwater and surface water based on the percentage TPH relative to total organic carbon (TOC) was developed. This technique can be used to indicate whether a sample contains polar compounds from NOM or petroleum, or whether more detailed analysis is required to identify the source.

Chapter 2

2 Compound class characterisation of a TPH groundwater plume associated with weathered diesel

Some of the work presented in this chapter has been published as:

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Abstract

High TPH concentrations (up to 13.0 mg/L, C15-C28 region) were observed in groundwater associated with long-term diesel contamination over a period of 12 years. Compound class characterisation of the TPH extracts demonstrated that polar components dominated the TPH plume, with aromatic components representing only a minor contribution. The identification of a polar compound (1-adamantanol) with no natural precursors suggested the polar components were formed from petroleum and not NOM. Compositional changes to the TPH plume with increasing distance downgradient from the source corresponded with an increased polar component, suggesting the polar compounds are relatively resistant to further degradation under the conditions at the site. No significant change in the UCM profile of the polar component was observed, also demonstrating a resistance to degradation. Because a majority of the polar components were present as a UCM it is difficult to assess the associated risk of these polar components.

2.1 Introduction

Petroleum released into the environment is typically subject to weathering processes that can significantly alter its chemical composition (Kaplan *et al.*, 1996). This includes the volatilisation of low molecular weight components, the dissolution of aromatic components, and biodegradation of aliphatic, aromatic and heterocyclic components (Christensen and Larsen, 1993; Kaplan *et al.*, 1996; Kaplan *et al.*, 1997). Though weathering processes such as biodegradation typically result in the depletion of petroleum constituents, biodegradation can also result in the formation of polar compounds as degradation products (Atlas, 1981; Gibson and Subramanian, 1984; Heider *et al.*, 1998; Fritsche and Hofrichter, 1999; Foght, 2008). Several degradation products can be formed from a single petroleum component, and therefore the number of compounds present and the complexity of the petroleum derived material can be greatly increased (Thorn and Aiken, 1998).

These compositional changes and the presence of polar compounds in soil and groundwater associated with weathered petroleum can make risk assessments difficult (Zemo and Foote, 2003; Lundegard and Sweeney, 2004). In some instances, polar compounds resemble a “hump” referred to as a UCM (Zemo and Foote, 2003) and the differentiation between petroleum and background NOM can be difficult (Girard and Edelman, 1994). As a result, NOM can be incorporated in TPH and result in elevated levels of TPH (Girard and Edelman, 1994). On the other hand, the removal of these compounds may underestimate the level of petroleum-derived contamination.

2.2 Scope of study

In this chapter, increasing TPH concentrations in groundwater associated with weathered diesel were investigated. Groundwater samples were analysed for TPH, and compound class characterisation performed to assess the proportion of aliphatic, aromatic and polar components present in the TPH. Changes to specific aliphatic and aromatic components in the TPH were also investigated and their fingerprint used to relate the TPH

downgradient of the expected source, as well as indicate specific changes due to weathering.

The site consists of an above-ground storage facility situated approximately 120 m from the Indian Ocean, on the Swan Coastal Plain south of Perth, Western Australia (Fig. 2.1). Storage tank releases of diesel are dated in excess of 50 years, resulting in a NAPL plume in the near water table zone with a maximum recorded thickness of 0.7 m (Johnston *et al.*, 2007). A detailed description of the site and weathered NAPL present can be found in Johnston *et al.* (2007).

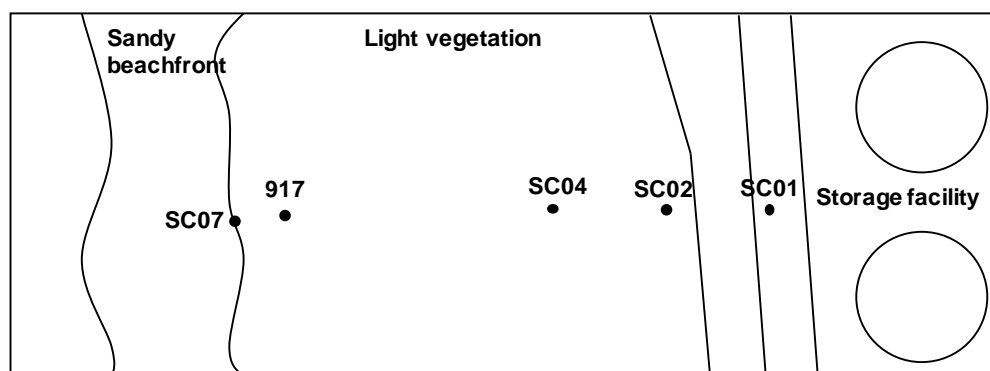


Figure 2.1 Topographical site map showing storage tanks and sampling bore locations.

2.3 Experimental

2.3.1 Sampling of groundwater samples

Groundwater (500 mL) samples for TPH (using GC-MS) and compound class characterisation were collected from monitoring wells downgradient of the above ground storage tank facility.

2.3.2 Total petroleum hydrocarbons (TPH)

TPH analysis of groundwater samples was carried out by spiking groundwater (250 mL) with a surrogate standard (d_8 -naphthalene, 5 μ g) prior

to extraction with dichloromethane (DCM; 10 mL) in a 500 mL separatory funnel. The DCM was dried with anhydrous sodium sulfate affording a sample ready for GC-MS analysis for C7 – C36 carbon range TPH. Concentration of the DCM extract by evaporation to 0.5 mL provided a sample ready for GC-MS analysis for the C10 – C36 carbon range TPH.

2.3.3 Compound class characterisation

Compound class characterisation of TPH extracts was carried out using a small-scale liquid chromatography method described by Bastow *et al.* (2007). The method has been shown to completely separate aliphatic, aromatic (including aromatic compounds containing up to five aromatic rings) and polar (compounds such as alcohols, acids, ketones and amines) components of petroleum. The method uses silica gel and various solvent mixtures to obtain fractions containing aliphatic, aromatic and polar components. Briefly, dissolved organic extracts were reduced to a small volume (approx. 10 μ L) and introduced onto the chromatography column. Aliphatic, aromatic and polar fractions were then eluted under gravity with *n*-pentane (1.8 mL), *n*-pentane/dichloromethane (7:3 v/v, 1.8 mL) and methanol/dichloromethane (1:1 v/v, 1.8 mL) respectively. All fractions eluted were ready for GC-MS analysis.

2.3.4 Gas chromatography mass spectrometry (GC-MS)

GC-MS analysis was performed using a Varian Saturn IV mass spectrometer interfaced with a Varian model 3400 gas chromatograph (GC). The GC was equipped with an Alltech AT-5ms column (30 m x 0.25 mm internal diameter column (AT-5 ms, Alltech). Samples were dissolved in DCM and injected (1 μ L) using a Varian 8100 autosampler fitted with a Varian 1093 septum-equipped programmable injector (SPI) on-column injector. Helium was used as a carrier gas with a constant pressure (typically 6 psi). The GC oven was temperature programmed from 35 °C (1 min) to 310 °C (10 min) at 5 °C/min. The ion trap was operated with an ionisation energy of 70 eV at 160 °C in scan mode (m/z 50-320).

2.3.5 Gas chromatography flame ionising detector (GC-FID)

Historical data (1995 – 2007) for groundwater TPH interpreted in this study was obtained from the National Measurement Institute (NMI) in Perth, Western Australia, where TPH was measured using a GC-FID.

2.4 Results and discussion

2.4.1 Total petroleum hydrocarbons (TPH)

Coastal groundwater located down gradient of an above ground storage facility was observed to have high TPH. A groundwater monitoring bore (917; Fig. 2.1) situated 103 m downgradient from the expected source showed a general increase in TPH, reaching a maximum concentration in 2005 (13.0 mg/L) that decreased in subsequent years (Fig. 2.2). TPH constituents were observed predominantly in the C15–C28 range (77% in 2005).

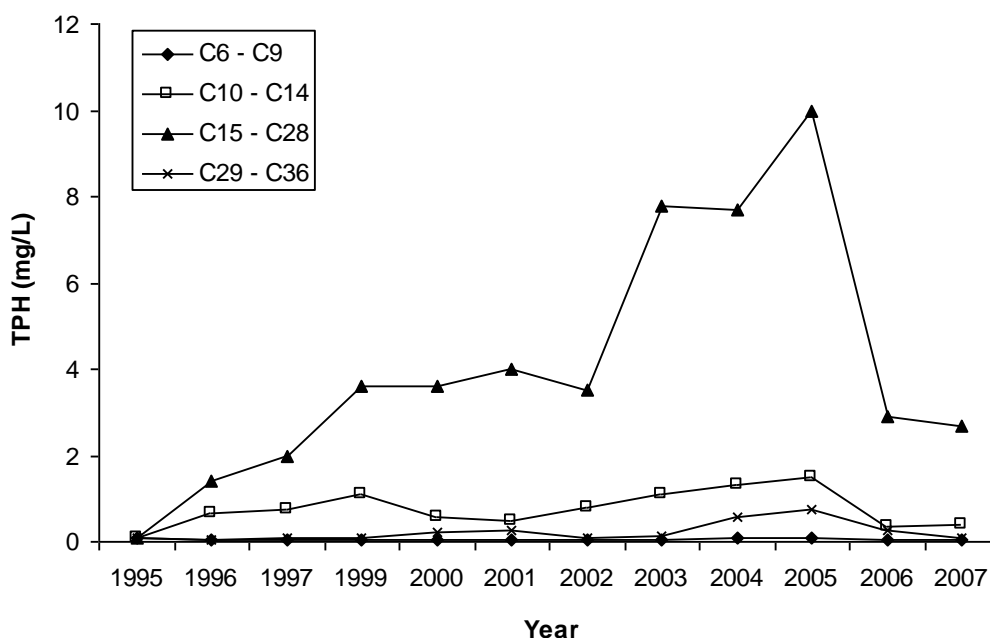


Figure 2.2 TPH concentrations at bore 917 from 1995 to 2007. Results obtained from NMI using GC-FID.

Groundwater sampled from a series of bores (SC01, SC02, SC04, and SC07; Fig. 2.1) downgradient of the expected source was analysed for TPH in July 2005. TPH measurements for these samples showed a general decrease in concentration with increasing distance downgradient (Fig. 2.3).

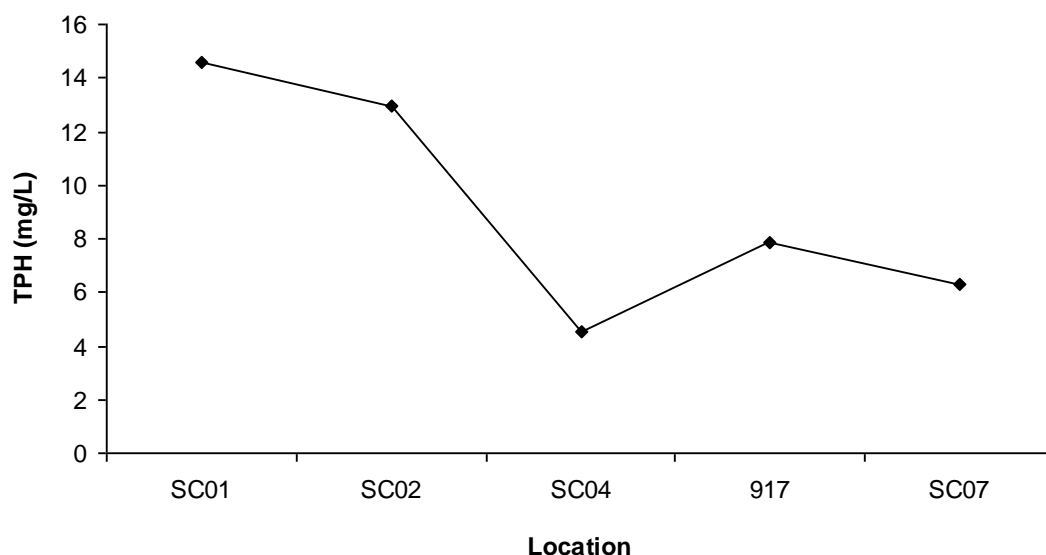


Figure 2.3 TPH concentrations for SC01, SC02, SC04, 917 and SC07 groundwater samples in 2005. Results obtained through GC-MS analysis.

Visual assessment of the TPH chromatograms for the samples shows some similarities and differences (Fig. 2.4). TPH chromatograms for almost all samples are dominated by a large UCM with resolvable components in the early eluting regions of the TPH profile (Fig. 2.4). TPH chromatograms for SC01 (Fig. 2.4a) and SC02 (Fig. 2.4b) have a similar appearance with many early eluting resolved components identified as alkylbenzenes, tetralins and naphthalenes. The TPH chromatogram for SC04 (Fig. 2.4c) also shows many early eluting resolved components, though these compounds have a higher relative abundance compared to the UCM. TPH chromatograms for 917 (Fig. 2.4d) and SC07 (Fig. 2.4e) show fewer early eluting resolvable components, with SC07 in particular dominated by a large UCM in the C15–C28 TPH region. All samples analysed contained this UCM, and it is likely to be the cause of increased TPH concentration in the C15–C28 region of sample 917 from 1995 to 2005.

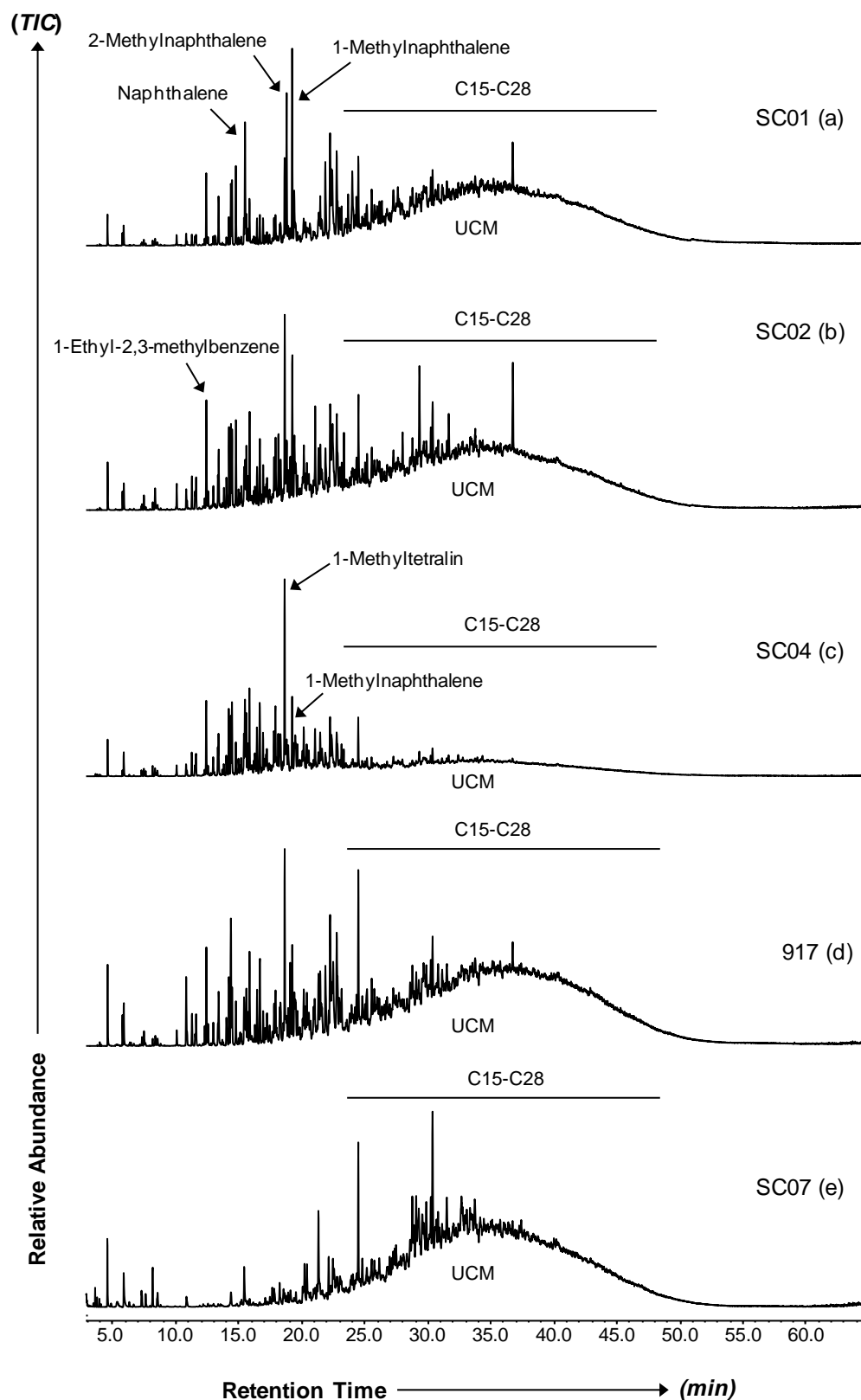


Figure 2.4 Total ion chromatograms (TIC) for TPH of a) SC01, b) SC02, c) SC04, d) 917 and e) SC07 groundwater samples, analysed using GC-MS. TICs are background subtracted for column bleed. UCM: unresolved complex mixture.

2.4.2 Compound class characterisation

Compound class characterisation was carried out to determine the proportions of aliphatic, aromatic and polar components in the TPH extracts (Table 2.1).

Table 2.1 Percentage of aliphatic, aromatic and polar fractions for groundwater TPH extracts.

Sample	Aliphatic fraction	Aromatic fraction	Polar fraction
SC01	0 %	6 %	94%
SC02	16 %	10 %	74 %
SC04	21 %	25 %	52 %
917	0 %	11 %	89 %
SC07	0 %	1 %	99 %

2.4.2.1 Aliphatic components

Aliphatic components were identified in the TPH extracts from bore SC02 and SC04, representing 16 and 21 % of the TPH respectively. These compounds should be present at negligible dissolved concentrations in water samples (aqueous solubilities for *n*-C15 and pristane are 0.04 µg/L and 0.01 µg/L respectively; Tolls *et al.*, 2002). This therefore indicates the presence of non-dissolved phase hydrocarbons as a result of NAPL sorbed to particulate material or micro-droplets (Lundegard and Sweeney, 2004). TICs for the aliphatic components of SC02 (Fig. 2.5b) and SC04 (Fig. 2.5c) show the presence of a UCM with pristane and phytane as dominant resolved compounds. This distribution of aliphatic components is typical for a biodegraded diesel where the *n*-alkanes have been removed, typically having a spill age greater than 20 years (Christensen and Larsen, 1993) and indicates that recent spill events are not likely to have contributed to the TPH.

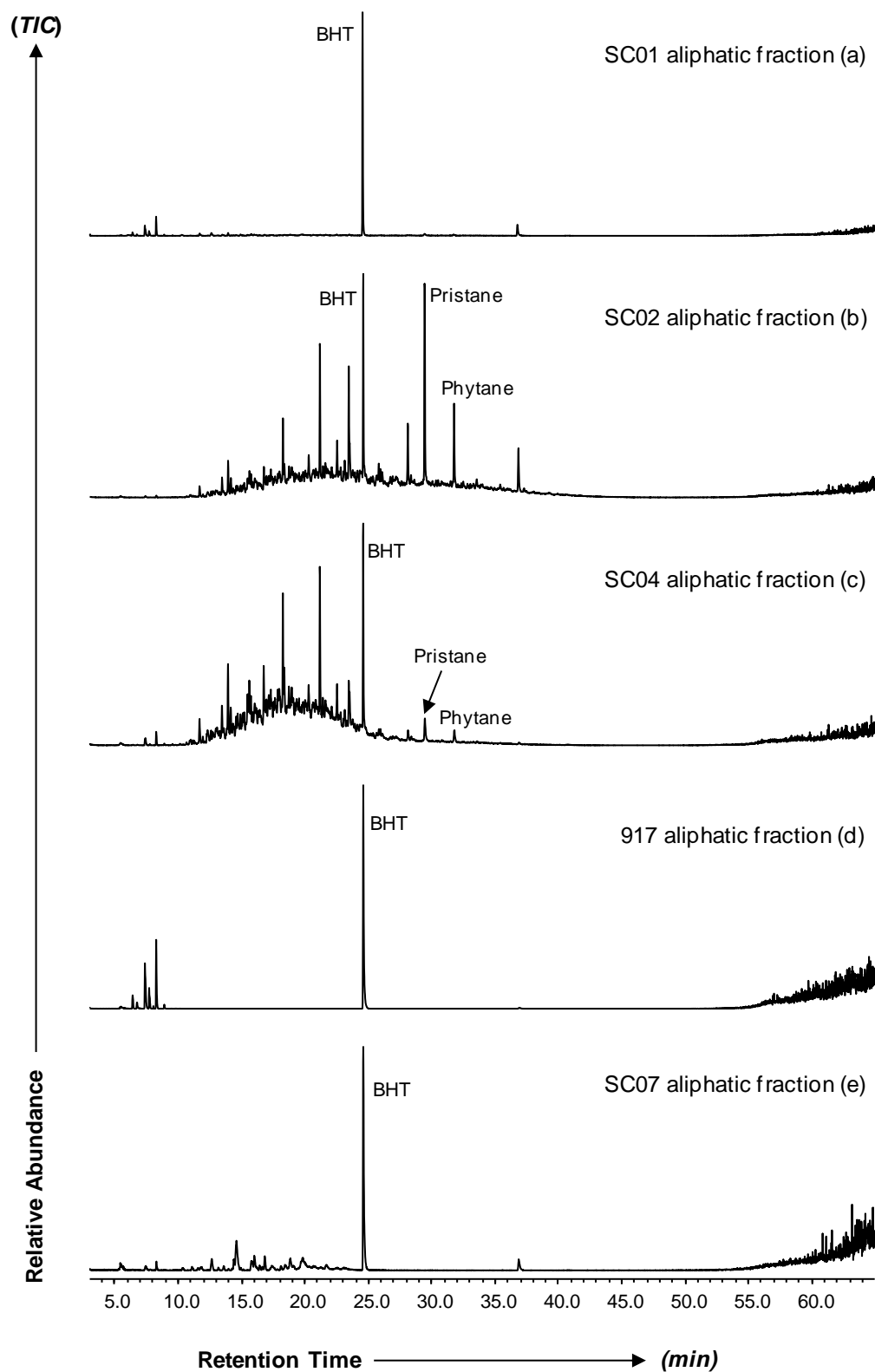


Figure 2.5 Total ion chromatograms (TICs) for the aliphatic fractions of a) SC01, b) SC02, c) SC04, d) 917 and e) SC07. TICs are background subtracted for column bleed. BHT: Butylated hydroxytoluene solvent preservative.

2.4.2.2 Aromatic components

Aromatic components were present in all TPH extracts (Fig. 2.6). Bore SC04 contained the highest proportion of aromatic components (25 %) which may be due to the presence of non-dissolved phase hydrocarbons (as indicated by the high proportion of aliphatics in this sample) or the result of a different NAPL source.

To indicate whether the TPH in the samples was derived from the same NAPL, groups of aromatic components that are relatively resistant to biodegradation (C4-alkylbenzenes and dimethylnaphthalenes) were investigated (Fig. 2.7 and 2.8). Comparison of the C4-alkylbenzene (Fig. 2.7a-e) distributions in the TPH extract with that present in an non-degraded crude oil (Fig. 2.7f) shows the samples have been affected by biodegradation. This is evident by the depletion of the generally abundant 1,2,3,5-tetramethylbenzene isomer and the relative enhancement of the 1,2,4,5- and 1,2,3,4-tetramethylbenzene isomers, which have likely been enhanced due to their higher resistance to biodegradation (George *et al.*, 2002). Comparison of the C4-alkylbenzenes in the TPH extracts of all samples show a pattern with remarkable similarities, except SC07 which has likely been affected by biodegradation to greater extent (depleted in 1-ethyl-2,4-dimethylbenzene). These similar, biodegraded signatures that are not common to crude oil (and likely other unaltered fuels) suggest the samples share the same source.

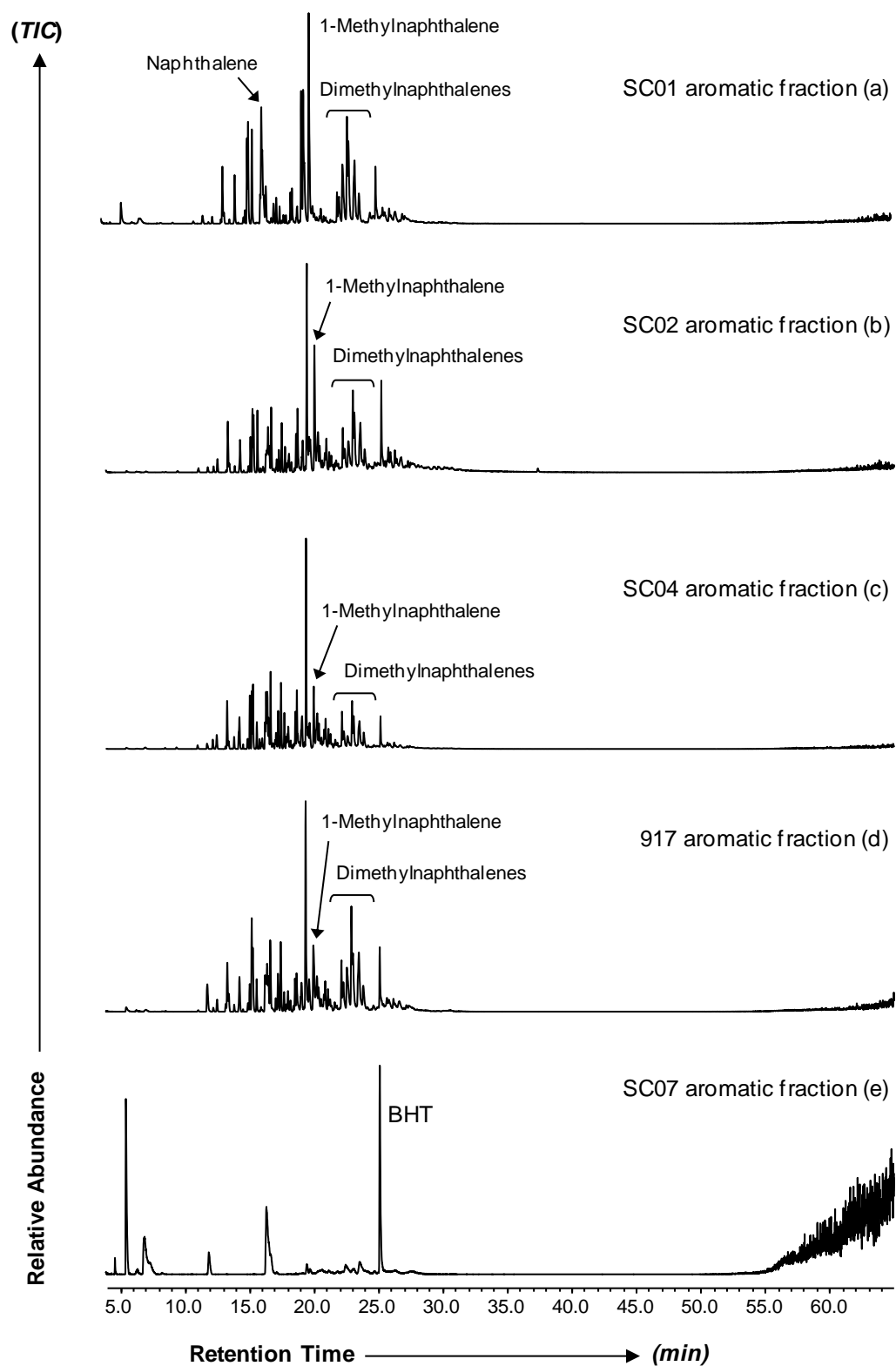


Figure 2.6 Total ion chromatograms (TICs) for the aromatic fractions of a) SC01, b) SC02, c) SC04, d) 917 and e) SC07. TICs are background subtracted for column bleed. BHT: Butylated hydroxytoluene solvent preservative.

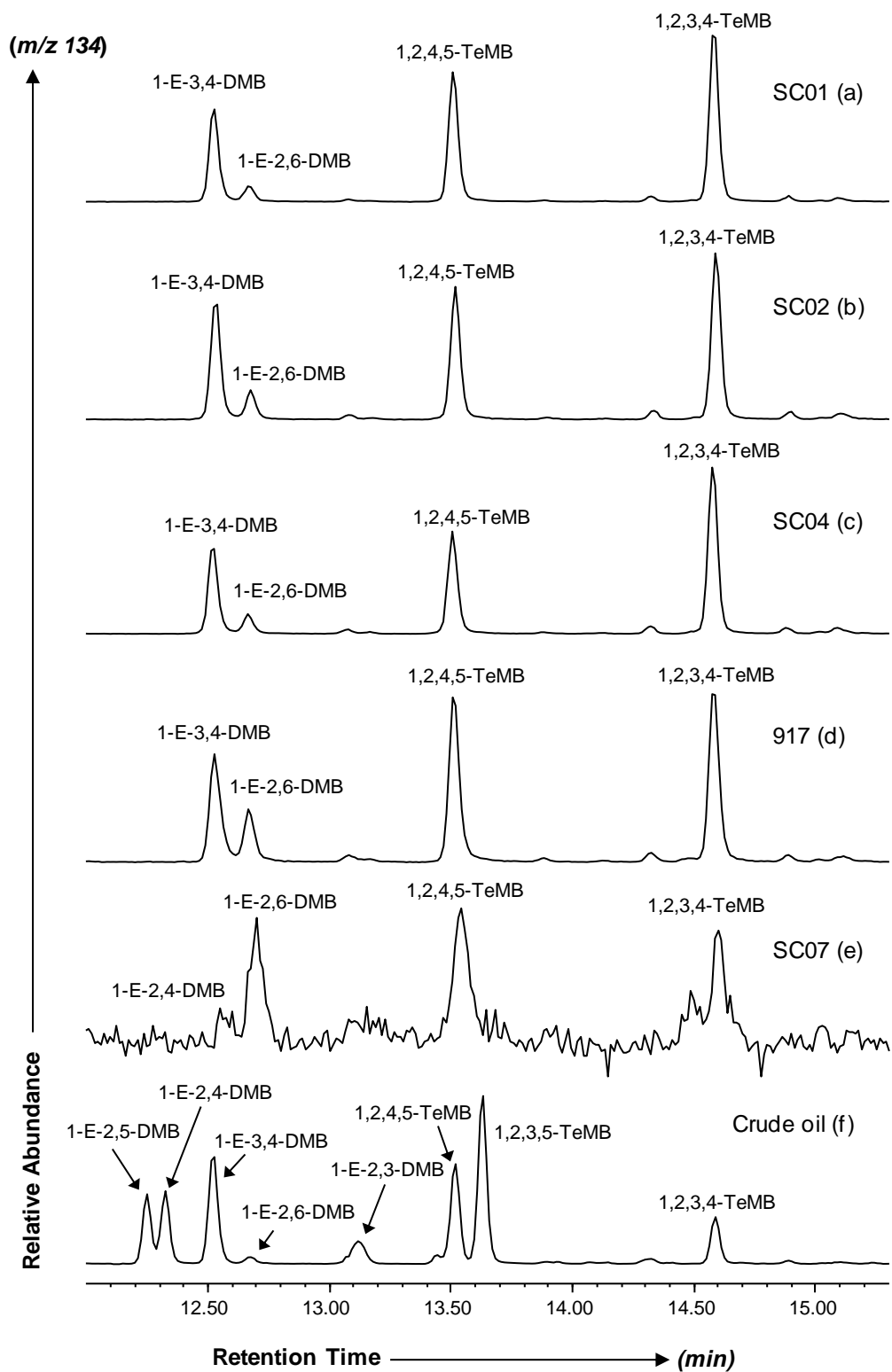


Figure 2.7 Partial ion chromatogram (m/z 134) showing the tetramethylbenzenes and ethyldimethylbenzenes for a) SC01, b) SC02, c) SC04, d) 917, e) SC07 and f) crude oil. E: Ethyl, DMB: Dimethylbenzene, TeMB: Tetramethylbenzene.

Changes to the distribution of dimethylnaphthalenes in the TPH (Fig. 2.8) can be used to show increased biodegradation with increasing distance from the source, and also show a general trend that provides further evidence the TPH is derived from the same source. The dimethylnaphthalene distributions in 917 (Fig. 2.8d) and SC07 (Fig. 2.8e) show a decrease in the relative abundance of 1,6-dimethylnaphthalene compared to TPH closer to the source. This suggests that biodegradation has resulted in an altered distribution in these samples, as the 1,6-configuration typically exhibits the highest susceptibility to biodegradation (Fisher *et al.*, 1998). Evidence of extensive biodegradation is shown at the greatest distance from the source, with the most susceptible isomers completely removed from SC07 (i.e. 1,6-, 2,7-, 2,6-, 2,7-, 1,7-, and 1,3-dimethylnaphthalene; Fisher *et al.* 1998).

The TPH extract of SC07 contains the lowest proportion of aromatic components (1%; Table 2.1). Comparison of C4-alkylbenzene distributions and the close proximity (10 m) of SC07 to 917 suggest the TPH of SC07 shared the same source as the groundwater samples closer to the suspected source. The removal of the most susceptible dimethylnaphthalene isomers to biodegradation in SC07 corresponds with the severely altered distribution of C4-alkylbenzenes, and a significant drop in the proportion of aromatic components (11 % in 917, 1 % in SC07). This suggests the aromatic component has been removed through biodegradation as the contaminant plume migrates downgradient.

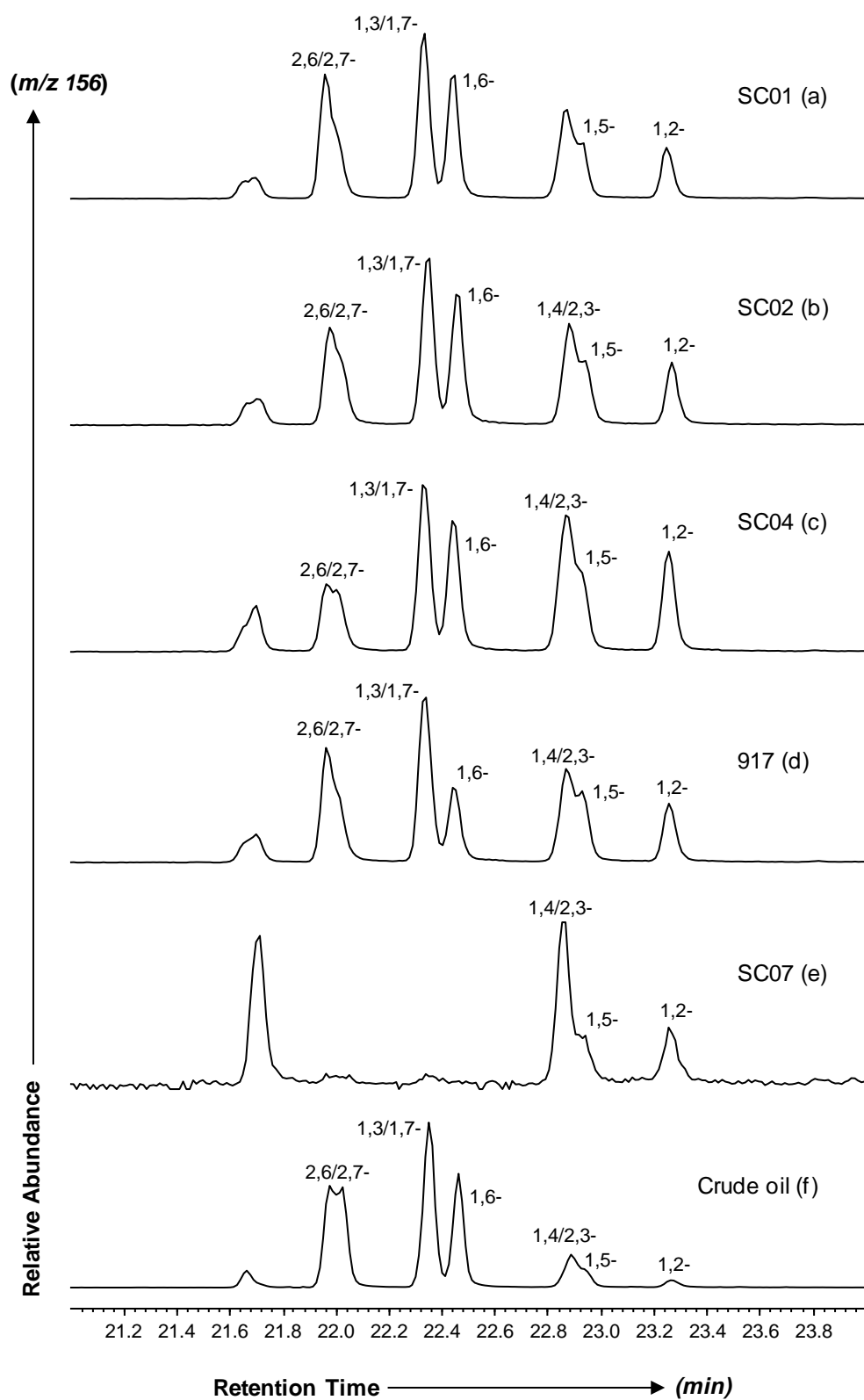


Figure 2.8 Partial ion chromatogram (m/z 156) showing the dimethylnaphthalenes for a) SC01, b) SC02, c) SC04, d) 917, e) SC07 and f) crude oil.

2.4.2.3 Polar components

Polar components were the major compound class present in all groundwater TPH without non-dissolved phase components (Table 2.1 and Fig. 2.5). The proportion of these polar components does not change significantly in TPH from the closest (94 %) and the furthestmost distance (99 %) from the source. TICs for the polar fractions contain large UCMs with a very similar profile (Fig. 2.9). This suggests that while dissolved hydrocarbons (i.e. aromatic components) have been significantly altered with increasing distance down gradient, the polar UCM that encompasses the majority of the TPH has remained relatively unchanged.

Sources for polar components in groundwater TPH associated with weathered diesel include polar compounds originally present in the crude oil used to produce the diesel; polar compounds formed from the degradation of weathered diesel and NOM (Zemo and Foote, 2003; Lundegard and Sweeney, 2004). Some polar compounds in the TPH could be identified, and were evaluated as potential markers that could indicate the likely source of the polar component. Adamantanols were identified (1-adamantanol identified using an authentic standard and methyladamantanols tentatively identified; Fig. 2.11) in the polar fractions of all the samples. These compounds contain a unique diamondoid carbon skeleton that can only be formed through high temperature and pressure processes that occur during crude oil formation or synthetic reactions (Mair *et al.*, 1959; Olah, 1990). Compounds with a diamondoid carbon skeleton have no known natural precursors, and in this instance are likely formed from the biodegradation of adamantanes originally present in the diesel NAPL.

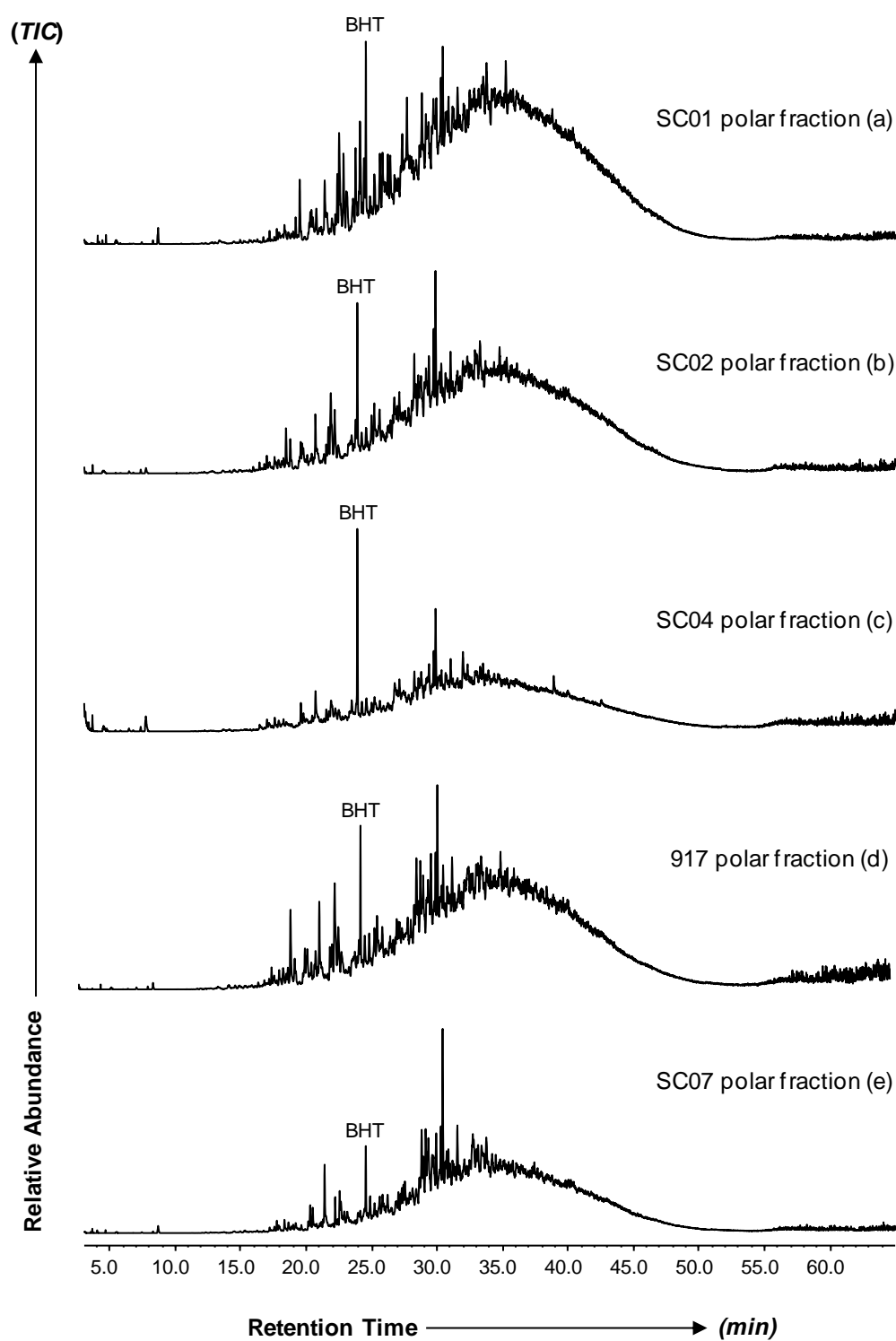


Figure 2.9 Total ion chromatograms (TIC) for the polar fractions of a) SC01, b) SC02, c) SC04, d) 917 and e) SC07. TICs are background subtracted for column bleed. BHT: Butylated hydroxytoluene solvent preservative.

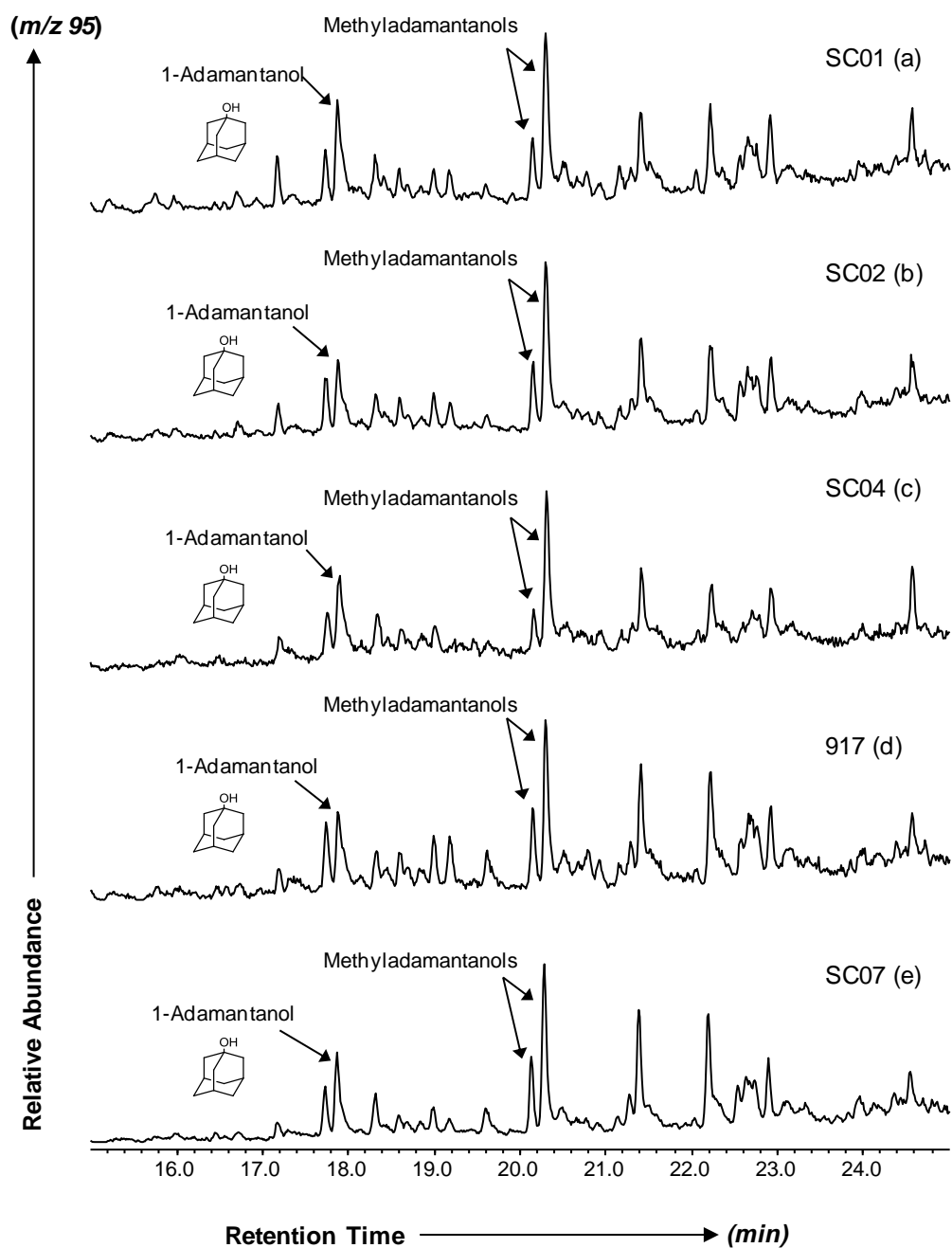


Figure 2.10 Adamantanol and tentatively identified methyladamantanols from the polar fractions of a) SC01, b) SC02, c) SC04, d) 917 and e) SC07.

The presence of adamantanols suggests that the polar component within the TPH extracts is at least partially derived from petroleum. Adamantanol distributions in the TPH of all the samples are remarkably similar, suggesting they are relatively resistant to biodegradation even at the greatest distance from the source where significant biodegradation was shown. Adamantanols are therefore potentially good markers to identify petroleum derived polar components present in TPH as a UCM. In addition, the similar distribution of adamantanols further suggests the samples originate from a common source.

2.5 Conclusions

High concentrations of TPH in groundwater were observed in a coastal groundwater aquifer associated with a weathered diesel that persisted over a 12 year monitoring period. TPH in the samples was present mainly as a large UCM consisting of polar components (89-99 %) based compound class characterisation. The polar UCMs in the samples were largely unaffected by natural attenuation processes compared to the aliphatic and aromatic components, which were depleted by biodegradation with increased distance from the likely source. The presence of 1-adamantanol and methyladamantanols indicated that at least some of the polar components in these samples originated from petroleum rather being present as a result of background NOM. The persistence of these polar components (which remain largely uncharacterised due to their presence as a UCM) and their effects on the environment and human health are unknown.

Chapter 3

3 Polar compounds from the dissolution of weathered diesel

Some of the work presented in this chapter has been published as:

Lang, D. A., T. P. Bastow, B. G. K. Van Aarssen, B. Warton, G. B. Davis & C. D. Johnston. 2009. Polar Compounds from the Dissolution of Weathered Diesel. *Ground Water Monitoring and Remediation*, 29, 85-93.

3.1 Abstract

The dissolution of hydrocarbon and non-hydrocarbon components from fresh and highly weathered diesels (spilled up to 50 years ago) from two sites were investigated. The fresh and weathered diesels were equilibrated with water using a slow stirring method, and the non-aqueous phase diesel and equilibrated aqueous water phase analysed using a range of analytical procedures. The water phase equilibrated with weathered diesels had higher total dissolved organics concentrations (96.0 and 8.6 mg/L at the two sites) compared to the water phase equilibrated with fresh diesel (average of 3.4 mg/L). Compound class characterisation of dissolved organics in water from the weathered diesel showed that polar components were a significant compound class (98 % and 42 % at the two sites) and appeared largely as UCM in the TICs. By definition, TPH analyses are aimed at measuring only dissolved hydrocarbon components, and dissolved polar compounds present as a UCM are often assumed to be NOM and removed using silica gel cleanup. This may result in a significant underestimation of the total soluble organic material in water associated with weathered diesels. In

addition, the environmental and/or human health risk posed by these fuel-derived polar compounds is unknown.

3.2 Introduction

The unintentional release of petroleum in the environment is an ongoing concern that can result in long-term contamination (Christensen and Larsen, 1993).

Petroleum that enters the subsurface can persist in the vadose zone and the near-water table zone long after the release event (up to 50 years) (Johnston *et al.*, 2002; Hamed, 2004). During this period, NAPLs can contaminate groundwater via the dissolution of NAPL constituents (Kim and Corapcioglu, 2003) and air via volatilisation (Davis *et al.*, 2005; Davis *et al.*, 2009). Petroleum fuels are complex mixtures, and in the subsurface environment undergo compositional changes over time due to volatilisation, dissolution and biodegradation (Christensen and Larsen, 1993; Kaplan *et al.*, 1996; Kaplan *et al.*, 1997; Kaplan *et al.*, 2001; Kaplan, 2003; Kim and Corapcioglu, 2003; Liu, 2004; Lundegard and Johnson, 2006).

Tracking changes in petroleum composition, determining parent and daughter product components, and quantifying the changes in risk in human health and the environment can be problematic. Recently, concerns have been raised about interferences in the TPH analyses of groundwater due to the presence of polar compounds. Polar compounds differ from hydrocarbons due to the incorporation of nitrogen, sulfur and oxygen atoms in the structure of the organic molecules (Tissot and Welte, 1984). TPH methods are aimed at analysing semi-volatile hydrocarbons, though polar compounds can also be incorporated, resulting in elevated TPH concentrations that are not representative of the actual level of hydrocarbon contamination (Zemo and Foote, 2003; Lundegard and Sweeney, 2004; Lang *et al.*, 2008). Sources of these polar compounds include compounds originally present in crude oil (Lundegard and Knott, 2001; Liu, 2004; Lundegard and Sweeney, 2004), fuel additives (Belpoggi *et al.*, 1995; Schmidt *et al.*, 2002b; White *et al.*, 2002), NOM (Girard and Edelman, 1994; Zemo and Foote, 2003; Lundegard and Sweeney, 2004) and metabolites from the biodegradation of hydrocarbons (Atlas, 1981; Cozzarelli *et al.*, 1990; Cozzarelli *et al.*, 1994; Beller *et al.*, 1995; Beller, 2000; Foght, 2008). Polar compounds from different sources can

be difficult to distinguish and differentiation cannot be accomplished by standard methods, such as EPA Method 418.1 (Girard and Edelman, 1994; Thorn and Aiken, 1998). Due to the problems associated with polar compounds and TPH, it has been suggested that a compound specific analysis should be used as an alternative to TPH, and/or the polar compounds should be removed from TPH extracts using silica gel clean-up (Zemo and Foote, 2003). However, to exclude polar compounds associated with fuel may underestimate the detrimental impact of petroleum on groundwater quality.

3.2.1 Scope of study

This chapter reports the accumulation of polar compounds in weathered diesel from two contaminated subsurface locations that partition into water and give elevated levels of dissolved organic compounds compared to water equilibrated with fresh diesel. The polar compounds are present as a UCM in the water phases of the weathered diesel making the source of the polar compounds difficult to identify. A marker compound is identified and used to show that at least some of the soluble polar fraction is derived from petroleum rather than from NOM.

3.3 Experimental

3.3.1 Diesel samples

Three diesel samples were selected for this study. A fresh diesel was obtained from a retail outlet, a weathered diesel (weathered diesel-1) was obtained from a spill release estimated to be up to 50 years old (the same site investigated in Chapter 2; Johnston *et al.*, 2007) and a second weathered diesel (weathered diesel-2) obtained from an anonymous site. Weathered diesel-1 was recovered in a large volume (~500 mL) by bailing a well with a typical product thickness of 0.7 m. The sample was sub-sampled to establish duplicate equilibration experiments. Weathered diesel-2 was obtained in a much smaller volume (~40 mL) from an anonymous site of contrasting hydrogeology to that of the first weathered diesel within the same regional setting, which had a spill age less than weathered diesel-

1. Only one duplicate equilibration experiment was carried out with weathered diesel-2 due to the quantity available.

3.3.2 Equilibration procedure

The dissolution of components of the diesels into water was studied in laboratory scale experiments. Partitioning of constituent components can occur through turbulent (Lee *et al.*, 1992) and non-turbulent (Schluep *et al.*, 2001) mixing of NAPLs and water. Water soluble components partition into the aqueous phase until equilibrium between both phases has been reached (de Bruijn *et al.*, 1989). The slow-stirring method (SSM) equilibration experiment used here is a common laboratory technique used to assess the partitioning of components from NAPLs into water (Schluep *et al.*, 2001) and the preparation of the water soluble fraction from petroleum fuels (Mahoney and Haskin, 1980). By stirring gently, the SSM promotes non-turbulent equilibrium of the NAPL and water phases, keeping the NAPL/water interface intact (de Bruijn *et al.*, 1989), as opposed to “shake flask” methods where vigorous mixing results in NAPL/water emulsions and micro-droplets in the aqueous phase (Shaw and Reidy, 1979; Schluep *et al.*, 2001). Micro-droplets of NAPL can lead to compounds of very low aqueous solubilities being measured in the water phase, even though they are not dissolved in the aqueous phase. This can result in a misrepresentation of the dissolved components in the water phase, resembling the profile of compounds in the NAPL phase (Shaw and Reidy, 1979).

Based on this, SSM equilibration experiments were carried out in 40 mL brown glass volatile organic analysis (VOA) vials (Fig. 3.1). A shortened Pasteur pipette with the thin glass end removed was fitted through a Teflon-lined silicone septum to protrude approximately 12 mm from the top of the septum.

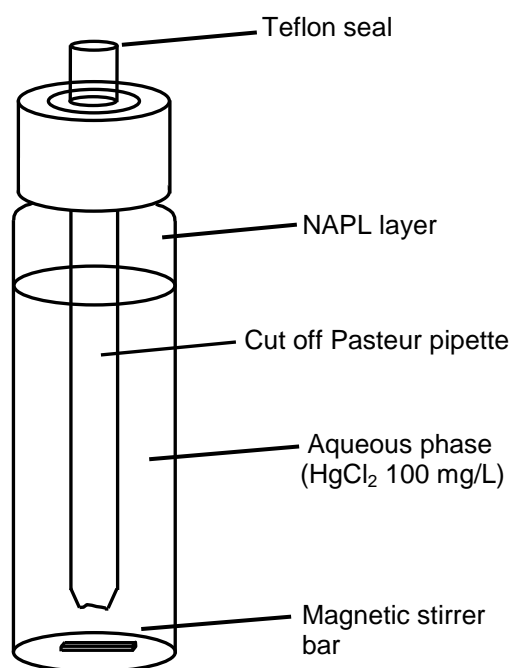


Figure 3.1 Diagram of diesel SSM equilibration experiment vessel

NAPL used in the equilibration experiments was placed on top of an aqueous mercuric chloride solution (30 mL, HgCl₂ 100 mg/L) in a brown glass vial (Fig. 3.1) containing a small magnetic stirrer bar (15 x 1.5 mm). Mercuric chloride was used as a microbial inhibitor in all the SSM experiments to avoid biodegradation of any component in the aqueous phase during the experiments. The lid was lifted approximately 1 cm while the NAPL was carefully introduced on top of the aqueous layer. The brown glass vial was then sealed and the top of the shortened Pasteur pipette sealed with Teflon. SSM equilibration experiments were carried out in duplicate using various volumes of NAPL (2.5 mL, 5 mL and 10 mL) and equilibration times up to 384 hr, along with duplicate procedural blanks. The stirrer bar rate was approximately 100 rpm for all experiments. SSM vials were stirred on heating mantles in a temperature-controlled room (25 °C) in large glass containers filled with water to reduce temperature fluctuations. Temperature fluctuations of the water bath were monitored with a WTW 330i pH/Temp meter (range of 1.80 °C and mean of 25.1 °C). Water for analysis was removed through the shortened Pasteur pipette with the lid partially unscrewed to allow for pressure equilibration.

3.3.3 Total petroleum hydrocarbons (TPH)

Dissolved organic compound concentrations from SSM equilibration experiments were measured as TPH. Water samples (19.2 mL) were acidified (0.3 mL, 4.5 M HCl) and spiked with a surrogate standard d_8 -naphthalene (5 μ g) prior to extraction with dichloromethane (DCM; 1 mL). After thorough mixing, the DCM layer was removed and dried using anhydrous sodium sulfate to provide a sample ready for GC-MS analysis. Quantification of the total dissolved organics (based on total ion chromatogram; TIC response) and individual compound concentrations (based on selected ion response) were performed by spiking known amounts of authentic standards into water and extracting them using the same procedure used for the water samples.

3.3.4 Compound class characterisation

Compound class characterisation of the dissolved organics extracts after initial GC-MS analysis was carried out using a method described by Bastow *et al.* (2007). The proportions of aliphatic, aromatic and polar components in the dissolved organics extracts were determined through GC-MS analysis using the procedure outlined in Chapter 2.3.3. In the case of the water samples from SSM equilibration experiments of fresh diesel, no components were observed in either the aliphatic or polar fractions. The total aromatic fractions only differed from the total dissolved organics extract in the proportions of some of the lower molecular weight components due to the solvent reduction step used to obtain the aromatic fraction. Therefore the dissolved organic fraction from the SSM equilibration experiments of the fresh diesel was deemed to contain only aromatic components. The dissolved organics in water samples from the SSM equilibration experiments of weathered diesels contained resolved peaks in the aromatic fractions and UCMs in the polar fractions. As a result, the proportions of aromatic and polar components were obtained by comparing the total dissolved organics and aromatic fraction which both contained the surrogate standard. The proportions of aliphatic, aromatic and polar components in the fresh and

weathered diesel samples were determined gravimetrically using a method described by Bastow *et al.* (2007).

3.3.5 GC-MS Analysis

Two GC-MS methods were employed in the analysis of samples. Method A used a GC phase that was restricted to a smaller carbon range compared with Method B, though enabled the identification of alkylnaphthalenes by established retention patterns. However, dissolved organic concentrations found with each method were comparable and the methods were therefore selected subject to availability.

For the GC-MS Method A, GC-MS analysis was performed using an Agilent 5975 MSD interfaced with an Agilent 6890 gas chromatograph equipped with a SGE BPX-5 column (30 m × 0.25 mm i.d., 1.0 µm film thickness). Samples were dissolved in dichloromethane and injected (1 µL) using a vaporising injector fitted to an autosampler. Helium was used as a carrier gas with a linear flow rate of 0.9 mL/min with the vaporising injector operated in pulsed splitless mode with an initial temperature of 290 °C and a pulse pressure of 15 psi at 0.5 min. The GC oven was temperature programmed from 35 °C (1 min), ramped to 60 °C at 5 °C/min and then ramped to 320 °C (10 min) at 15 °C/min. The MSD was operated with an ionisation energy of 70 eV at 230 °C in scan mode (m/z 50-550).

Differences in GC-MS Method B include the use of an Alltech AT-1 ms column (30 m × 0.25 mm i.d., 0.25 µm film thickness) and the oven temperature programmed from 35 °C (2 min) to 320 °C (10 min) at 15 °C/min.

3.3.6 GC-FID Analysis

Whole oil analysis of the fresh and weathered diesel was performed using a gas chromatograph coupled with a flame ionizing detector (GC-FID). This allowed detailed analysis of the components in the C₄-C₄₀ carbon range without the use of a solvent. Whole oil analysis was performed using a Hewlett Packard 5890A gas chromatograph equipped with an Alltech EC-1 column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Samples were injected neat (0.5 µL) using a vaporising

injector fitted to an Agilent 7673A autosampler. Hydrogen was used as a carrier gas with the vaporising injector operated in split mode. The oven was temperature-programmed to initiate at -10 °C (0 min) and ramped to 350 °C (4 min) at 10 °C/min (Schmidt *et al.*, 2003).

3.4 Results and discussion

3.4.1 General compositional differences between the fresh and weathered diesels

The dominant processes of degradation can be inferred by describing specific losses of compound groups and individual compounds within these groups. For example, whole oil analysis (using a GC-FID) for the fresh and weathered diesels (Fig. 3.2) show a loss of *n*-alkanes in the weathered diesels, with the relatively biodegradation resistant isoprenoids pristane and phytane remaining. This is typical for biodegraded mid-range distillates of petroleum with residence times in a soil profile of over 20 years (Christensen and Larsen, 1993) and suggests no significant input from any recent spill.

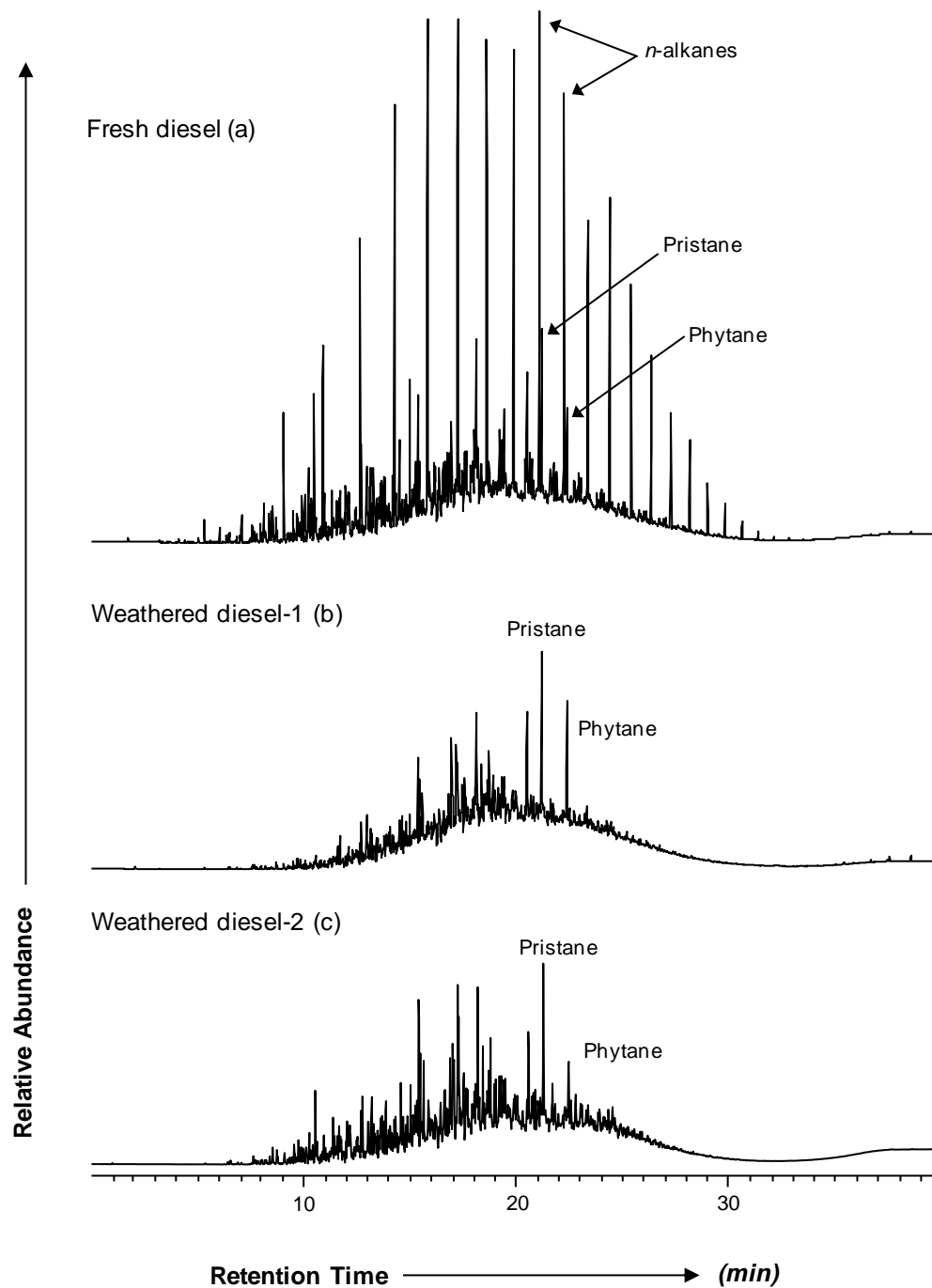


Figure 3.2 GC-FID chromatograms for the whole oil analysis of a) fresh diesel, b) weathered diesel-1 and c) weathered diesel-2.

Concentrations of selected aromatic compounds from fresh diesel and weathered diesel-1 are shown in Figure 3.3. Benzene, toluene, ethylbenzene and xylenes

(BTEX) are present in low concentrations in weathered diesel-1 compared to a fresh diesel. This is expected considering lower molecular weight aromatic compounds are the most susceptible to evaporation, dissolution and biodegradation (Christensen and Larsen, 1993). In contrast, higher molecular weight aromatic components such as naphthalene and alkylnaphthalenes are present in higher concentrations in weathered diesel-1 compared to the fresh diesel. This is likely due to these compounds being less susceptible to evaporation, dissolution and biodegradation.

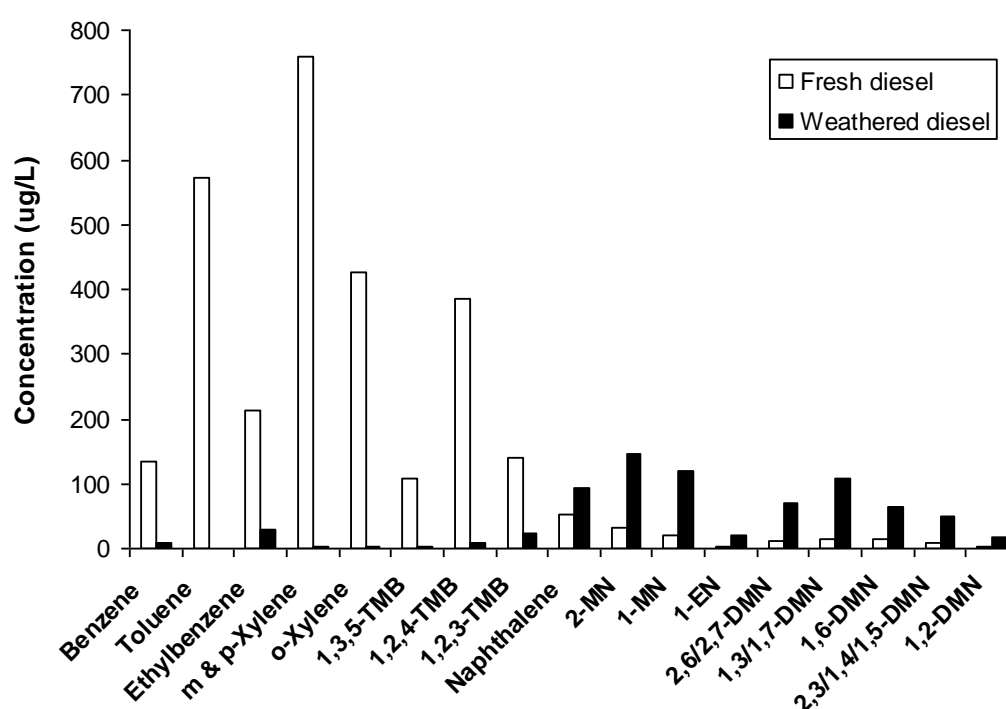


Figure 3.3 Concentrations of aromatic constituent components in fresh diesel and weathered diesel-1. Results reported using GC-MS method B. TMB: trimethylbenzene, MN: methyl naphthalene, EN: ethylnaphthalene, DMN: dimethylnaphthalene.

Comparisons of the relative abundance of individual compounds within the aromatic components indicate that weathered diesel-1 has undergone biodegradation, as some compounds that have similar physical properties (e.g. aqueous solubility) have varied distributions that are indicative of biodegradation. For example, ethylbenzene is more resistant to biodegradation than the xylenes (Wilkes *et al.*, 2000) and is the dominant BTEX compound in weathered diesel-1,

even though the solubility of this compound is comparable to the xylenes [solubility of ethylbenzene 0.161 g/L; xylenes 0.161 - 0.181 g/L at 25 °C; (CRC, 2007-2008)], which have been largely removed. Similarly, 1,2,3-trimethylbenzene is the dominant trimethylbenzene isomer in weathered diesel-1, compared with 1,2,4-trimethylbenzene being dominant in the fresh sample, the latter of which is more susceptible to biodegradation (George *et al.*, 2002).

These general compositional differences cannot be proven to have entirely occurred as a result of biodegradation of the diesel after its release, as the crude oil source and refining processes used may have differed. However, considering weathered diesel-1 and weathered diesel-2 have spill ages that are expected to be greater than 20 years, it is likely that at least some of these compositional differences between the fresh and weathered diesels are a result of biodegradation after its release.

3.4.1.1 Compound class characterisation of diesel NAPLs

Separation of fresh and weathered diesel into aliphatic, aromatic and polar components (Table 3.1) showed that polar components were undetectable in the fresh diesel. This is expected considering many polar compounds that are originally present in crude oil are likely to be removed during refining. Supporting evidence for this is the low colouration of fresh diesel compared to crude oils. Polar components were not present in the fresh diesel and their presence in the weathered diesels could be due to a number of sources. This includes NOM originally present in the soil or accumulated as biomass, and products from the degradation of diesel.

Table 3.1 Proportions of aliphatic, aromatic and polar components in fresh diesel, weathered diesel-1 and weathered diesel-2. Results are reported as an average of duplicate separations measured gravimetrically.

Compound class	Fresh diesel	Weathered diesel-1	Weathered diesel-2
Aliphatic fraction	76 %	62 %	53 %
Aromatic fraction	24 %	34 %	45 %

Polar fraction	0 %	4 %	2 %
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3.4.2 Equilibration experiments

3.4.2.1 Optimisation

Equilibration experiments were optimised to establish the optimal volume of diesel and time required to reach equilibration. These optimisation were carried out on fresh diesel and weathered diesel-1, but were not carried out using weathered diesel-2 due to the limited amount of NAPL available.

3.4.2.1.1 Diesel volume

The optimal volume of diesel required for the equilibration experiments was determined by increasing the volume of diesel used until the concentration of dissolved organics in the water reached a maximum (Fig. 3.4). The minimum volume of fresh diesel tested (2.5 mL of diesel per 30 mL of water) showed no significant depletion, with concentrations of dissolved organic compounds similar at each volume tested. In contrast, the optimum volume of weathered diesel-1 was likely to be more than the maximum volume tested (10 mL of diesel per 30 mL of water). However, even though concentrations of dissolved organics were still increasing, they appeared to be asymptoting to a maximum value. This difference in fresh diesel and weathered diesel-1 volume optimisations is possibly due to the presence of a large number of compounds that have relatively high water solubilities at low concentrations in the weathered diesel (e.g. a polar UCM). These components are more likely to be depleted from the NAPL compared to the BTEX components dominating the fresh diesel, which are present in relatively high individual concentrations in the NAPL but have lower relative water solubilities. As the ratio of NAPL to water in the 10 mL equilibration experiments were considered high and only a limited amount of the NAPL was available, 10 mL was used for all further equilibration experiments.

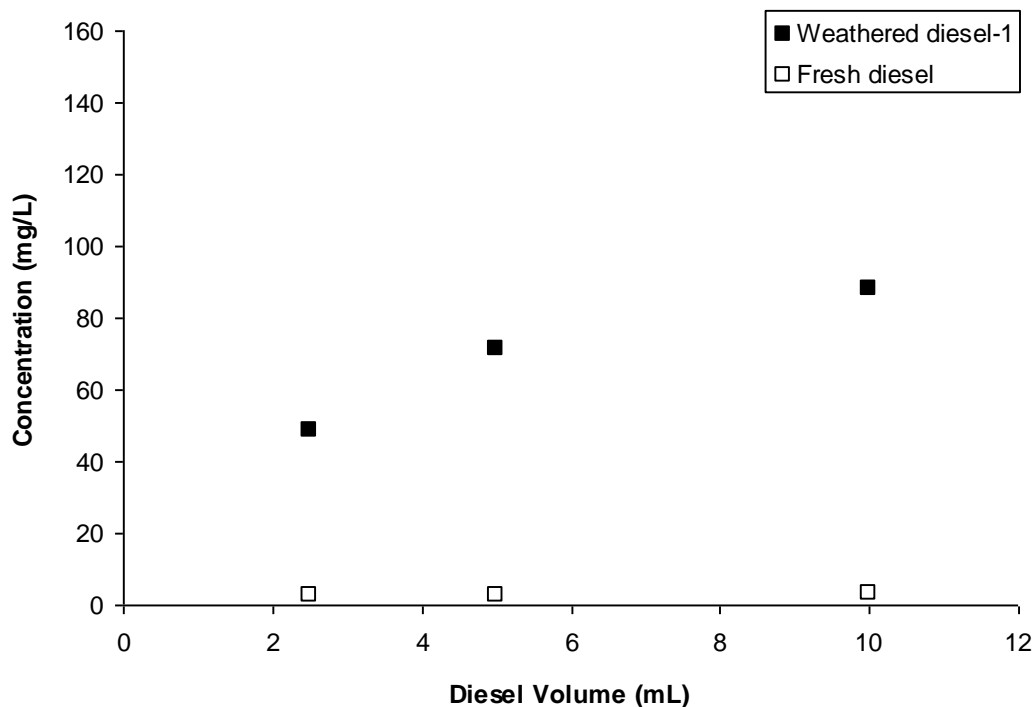


Figure 3.4 Dissolved organics concentrations in the water phase for fresh diesel and weathered diesel-1 equilibration experiments with different diesel volumes. Results reported as average of duplicate analyses using GC-MS Method A.

3.4.2.1.2 Equilibration time

Equilibrium experiments were carried out over a period of 384 hrs to determine the optimal time necessary to reach equilibrium. Analysis of the dissolved organics in the water phase obtained for fresh diesel experiments (Fig. 3.5a) showed that equilibrium was reached prior to 8 hrs, (average of 3.4 mg/L). Equilibration of the water phase in weathered diesel-1 experiments (Fig. 3.5b) was reached within 24 hrs with average total concentrations of dissolved organics after this period found to be 96 mg/L.

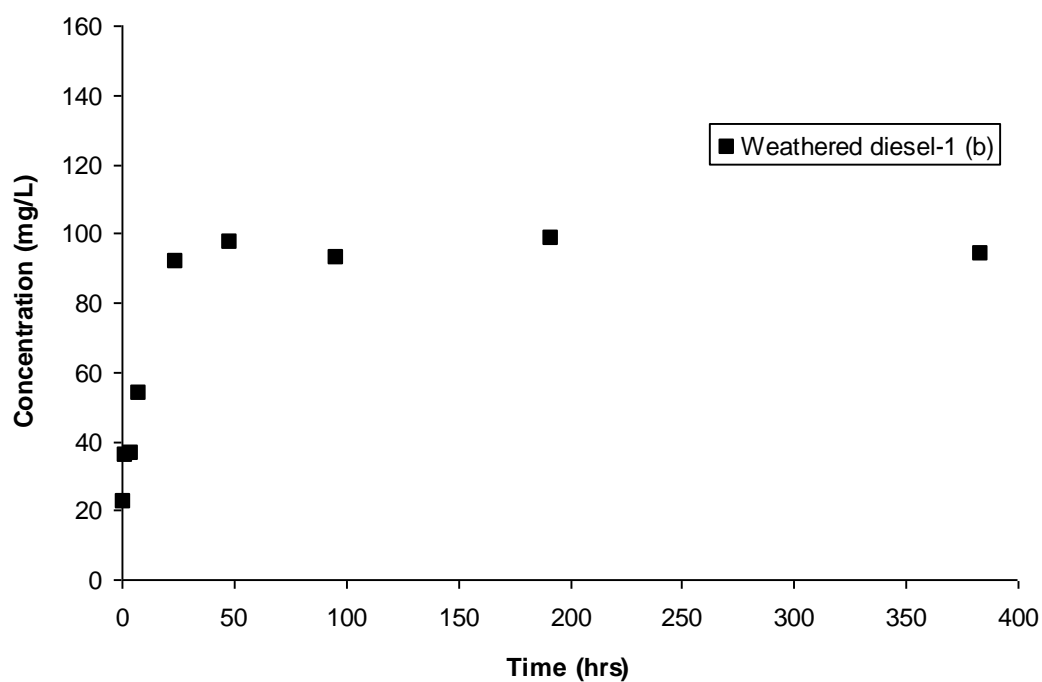
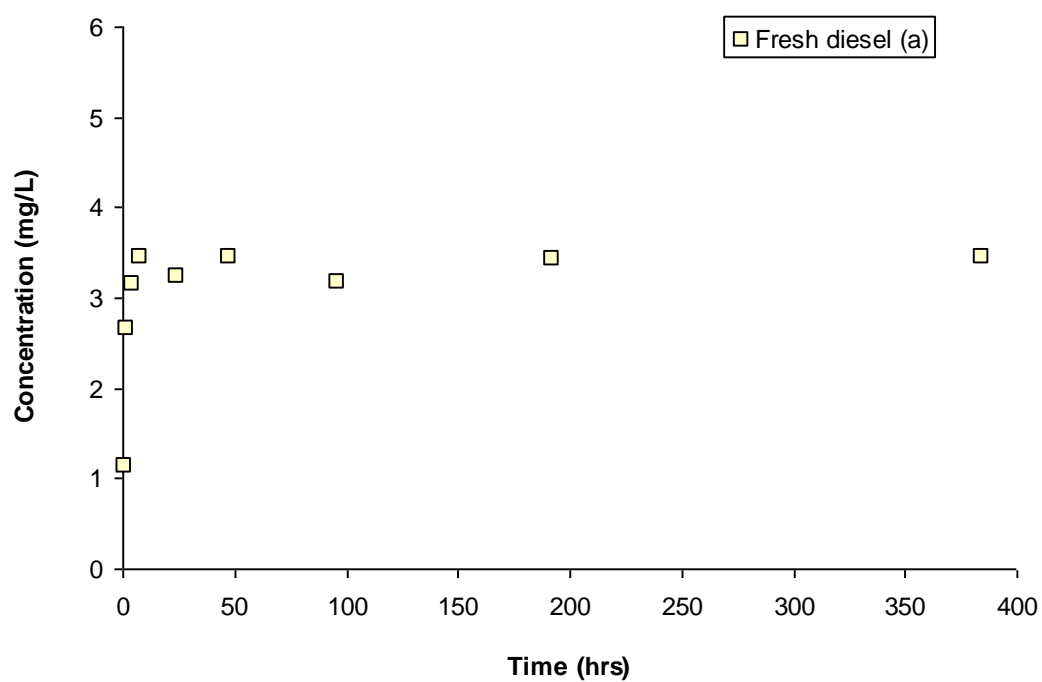


Figure 3.5 Concentrations of dissolved organics in the water phase for a) fresh diesel and b) weathered diesel-1 equilibration experiments over a 384 hr period. Results are reported as average of duplicate analyses using GC-MS Method A.

3.4.2.2 Dissolved organics concentrations

A much higher concentration of dissolved organics was observed in the equilibrated water phase of the weathered diesel-1 compared to the equilibrated water phase of the fresh diesel. Average concentrations in the water phase at equilibrium were 3.4 mg/L (range of 3.2 – 3.5 mg/L) and 96 mg/L (range of 92 - 98 mg/L) for fresh and weathered diesel-1 respectively. The concentration of dissolved organics in the equilibrated water phase of weathered diesel-2 for a single, 8 day measurement was 8.6 mg/L. As weathered diesel-1 has a greater spill age than weathered diesel-2, it suggests a greater spill age and level of biodegradation corresponds to increased concentrations of dissolved organics.

3.4.2.3 Composition of dissolved organics

TICs for dissolved organics from the water phases of fresh and weathered diesels (Fig. 3.6) show large differences in their compositions and molecular weight distributions. The components from the equilibrated water phase of fresh diesel (Fig. 3.6a) are easily resolved compounds eluting in the C7-C14 region. The water phase of weathered diesel-1 (Fig. 3.6b) consists predominantly of a UCM in the C15-C28 region, commencing prior to *n*-C14 and reaching a maximum in the C18-C21 region. Several partially resolved peaks are also present on this UCM, though these peaks could not be identified due to interferences from underlying components. The TIC for the dissolved organics from the water phase of weathered diesel-2 (Fig. 3.6c) shows features of both the fresh and the weathered diesel-1 samples, with resolved compounds in the C7-C14 region and a UCM in the C15-C28 region.

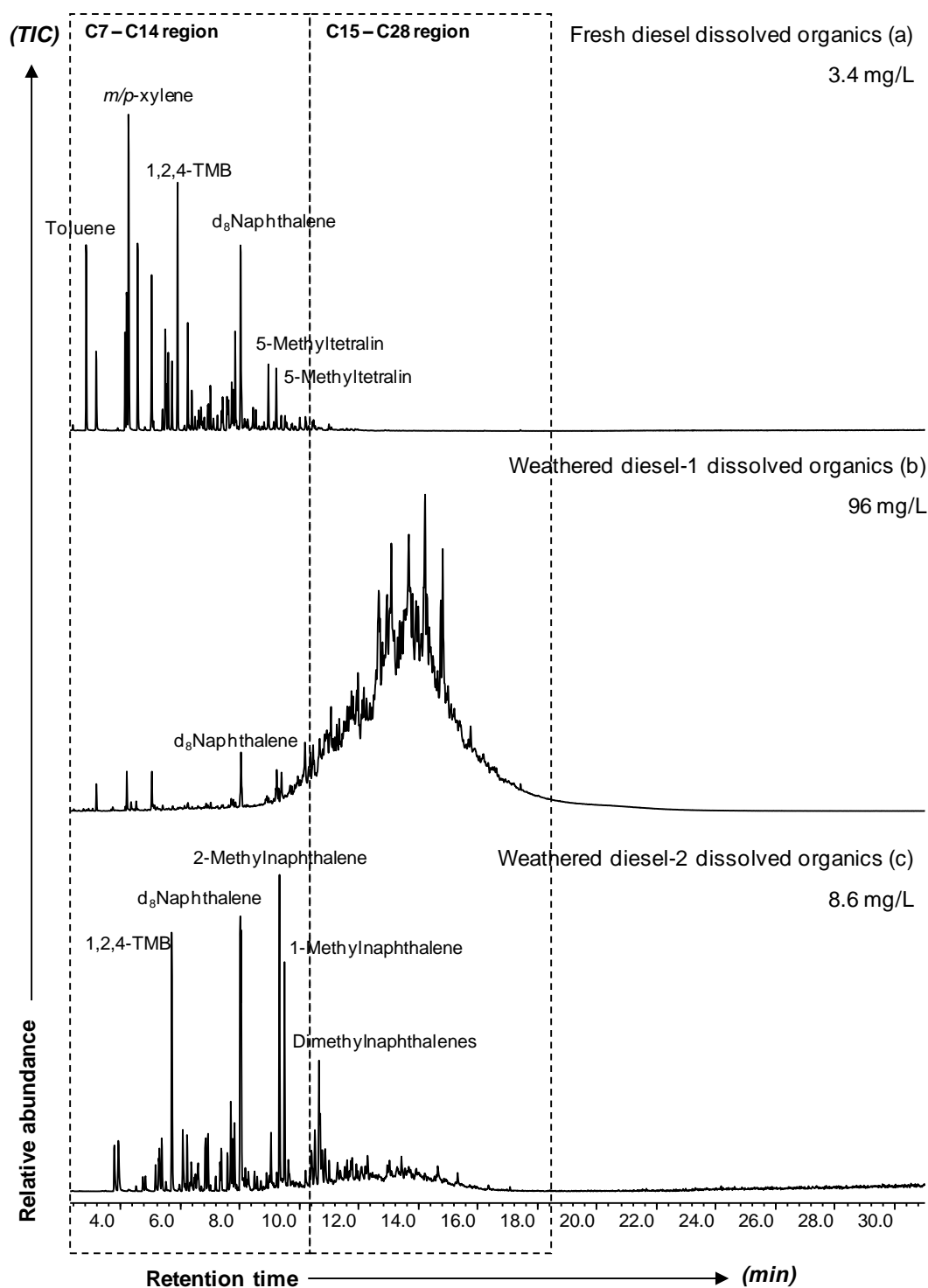


Figure 3.6 Total ion chromatograms (TICs) for dichloromethane (DCM) extracted dissolved organics from the water phase of a) fresh diesel, b) weathered diesel-1 and c) weathered diesel-2 equilibration experiments using GC-MS method B. TMB: trimethylbenzene.

3.4.2.3.1 Compound class characterisation

Separation of dissolved organics from the equilibrated water phase of fresh and weathered diesels into aliphatic, aromatic and polar fractions showed significant compositional differences (Table 3.2). Aliphatic hydrocarbon components are absent from the dissolved organics for both fresh and weathered diesels, which is likely a result of their negligible water solubilities. Aromatic compounds were identified as the dominant components (predominantly alkylbenzenes, alkyltetralins and alkylnaphthalenes) of the dissolved organics from the fresh diesel, with the polar components making no detectable contribution. In contrast, the organics in the water phase from the weathered diesel-1 contained 98 % polar and 2 % aromatic components. Dissolved organics from weathered diesel-2 contained a higher proportion of aromatic components (58 %) and a lower proportion of polar components (42 %) compared to weathered diesel-1.

Table 3.2 Proportions of aliphatic, aromatic and polar components in the water phase of fresh and weathered diesels. Results are reported as an average of equilibrium experiments (48 to 392 hrs) using GC-MS method A.

Compound class	Fresh diesel water phase	Weathered diesel-1 water phase	Weathered diesel-2 water phase
Aliphatic Fraction	0 %	0 %	0 %
Aromatic Fraction	100 %	2 %	58 %
Polar Fraction	0 %	98 %	42 %

The polar components in the weathered diesel NAPLs represented relatively low proportions (Table 3.1) in comparison to the dissolved organics from equilibrated water phases. This suggests that the higher water solubility of the polar components has resulted in strong partitioning into the water phase relative to the other compound classes. Therefore, the presence of more water-soluble components in weathered diesels can account for the large difference between the dissolved organics from fresh and weathered diesel. This shows that weathered diesel may contribute higher concentrations of dissolved organics to groundwater than fresh diesel. The higher proportion of

polar components in the dissolved water phase of weathered diesel-1 compared to weathered diesel-2 suggests that a greater spill age, and subsequently higher level of weathering corresponds to an increased potential for polar components to be imparted to water, and this may be reflected in spill events associated with weathered petroleum.

Sources for the polar components may include degradation products (abiotic and biotic) from the diesel, residual microbial biomass from degradation processes, NOM originally present in the soil, or polar compounds from the crude oil used to produce the diesel. The polar compounds from weathered diesel-1 may be sourced from NOM originally present at the site however the soil contains a very low soil organic matter fraction (Johnston *et al.*, 1998). Polar compounds from the crude oils used to produce the diesel may also be a potential source of the water soluble polar components in the weathered diesel equilibration experiments. However, as the weathered diesels are over 20 years in age, fresh samples are unobtainable and the original polar content is unknown. The fresh diesel used in this study contained no observable amounts of polar components and imparted no observable amounts of water soluble polar components to the equilibrated water phase. Weathered diesel-1 on the other hand, contained significant amounts of water soluble polar components that were depleted in the NAPL of weathered diesel-1 equilibration experiments at relatively high NAPL to water ratios. If these water soluble polar components were originally present within weathered diesel-1 before its release they are also likely to have been depleted through dissolution processes since the diesel has been in contact with groundwater for over 20 years. As a result, the enduring presence of these water soluble polar components within weathered diesels suggests they are continually forming and accumulating at a higher rate than they are being depleted from the diesel in the subsurface.

The polar compound 1-adamantanol was identified in the water phases of weathered diesel-1 and weathered diesel-2 with equilibrium experiments, with concentrations of 3.6 µg/L and 0.3 µg/L respectively. The presence of 1-adamantanol containing a diamondoid carbon skeleton suggests that at least

some of the polar components are formed from degradation of the weathered diesels. 1-Adamantanol is a compound that is likely to form through the oxidation of adamantane or adamantanes and has also been observed in the groundwater associated with weathered diesel (Lang *et al.*, 2008). Compounds with the adamantane carbon skeleton are ubiquitous in crude oils (Mair *et al.*, 1959), formed through high temperature and pressure processes that occur during its formation (Olah, 1990). 1-Adamantanol has no known natural precursors, and as a result is a potential marker for identifying the presence of petroleum-derived polar compounds in water. Its presence in the dissolved organic fractions from weathered diesel equilibration experiments therefore suggests that at least some of the polar components have formed from the degradation of diesel.

Polar compounds formed from the degradation of hydrocarbons have been documented previously from petroleum (Cozzarelli *et al.*, 1994; Barcelona *et al.*, 1995; Beller *et al.*, 1995; Langbehn and Steinhart, 1995). In some cases the metabolites of BTEX, including aromatic carboxylic acids and alcohols have been identified within groundwater associated with petroleum contamination. Polar components in the water phase of equilibration experiments in this study have accumulated in the diesel. This is surprising considering these compounds are significantly water soluble and would be expected to form near a water body (the microorganisms that are responsible for this biodegradation are likely to be present in the water phase). As a result, these compounds would be expected to be easily depleted from the NAPL rather than accumulating, as demonstrated through the optimisation of equilibration experiments in this study. The mechanisms by which these polar components form and accumulate in the NAPL could include aerobic degradation (abiotic and biotic) where the NAPL is in contact with air, and possibly anaerobically where the NAPL is in liquid-saturated intervals of the profile (Lundegard and Johnson, 2006). Davis *et al.* (2005) have indicated aerobic biodegradation of NAPL petroleum hydrocarbons occurs within a limited interval of the profile at the top of the capillary fringe. This is supported by more qualitative observations of highly degraded (non-petroleum fuel) organic material spread in the vicinity of the water table (Johnston and Patterson, 1994; Johnston *et al.*, 2007). Johnston *et al.* (2007) also showed corresponding vertical stratification of biodegradation markers in the NAPL. Despite the persistence of

vertical stratification of these indicators of the extent of biodegradation, water table fluctuations and consequent vertical displacement of a fraction of the NAPL would contribute to vertical mixing within the NAPL. This would be more pronounced at higher NAPL saturations because of the greater mobility of the NAPL in the profile. Vertical water movement may also account for dissolution and repartitioning of soluble species such as polar compounds. The accumulation of the polar compounds may therefore depend on a number of factors including groundwater flow, rainfall recharge, NAPL thickness and saturation, water table fluctuations and the extent of weathering.

3.5 Conclusions

The equilibration of two weathered diesels with water produced higher concentrations of dissolved organics in the water phase (96 mg/L and 8.6 mg/L) than equilibration of fresh diesel with water (3.4 mg/L). Speciation of the organics from the water phase of one weathered diesel showed that polar components in the C15-C28 carbon range were the major compound class (98 %) but were absent in the water phase organics equilibrated with fresh diesel. Equilibration of a second weathered diesel with water also provided elevated concentrations of organics in the water phase (8.6 mg/L) and a similar polar UCM (48 %) in the C15-C28 carbon range. The presence of a petroleum-derived metabolite 1-adamantanol in both weathered diesels suggests that at least some of the polar components in the weathered diesels are likely to have formed as a result of petroleum degradation. A microbial biomass or soil NOM cannot be eliminated as possible sources of some of the polar components because the polar UCM cannot be adequately characterised.

These findings highlight the ambiguity of groundwater analyses attempting to characterise the extent of contamination arising from weathered petroleum products, where GC-resolvable components may not be the major analytes. In the cases studied here, BTEX compounds and other analytes commonly associated with diesel were found to be only a fraction of the soluble organics dissolving from weathered diesel. This demonstrated that routine selected compound measurements and TPH measurements where polar compounds are removed

with silica gel clean-up may significantly underestimate petroleum contamination. There is evidence that some of the polar components in the weathered diesels used in this study are likely formed from the degradation of the original petroleum source, and there is a compelling argument to include dissolved polar compounds in the assessment of petroleum-fuel contaminated groundwater. Additionally, the toxicity of weathered diesel has been largely unexplored, and the large number and variety of metabolites that can be formed have not been assessed. Further research on the UCM and the polar compound species present within this UCM is required to ensure a potential toxicological risk is not being overlooked.

Chapter 4

4 Polar compounds in water and NAPL associated with weathered diesel

4.1 Abstract

A range of polar compounds were identified (some previously unidentified) in groundwater associated with weathered diesel and water equilibrated with weathered diesel. Compound types identified included alcohols, phenols, ketones and carboxylic acids. The carbon skeletons of these compounds related to some common petroleum constituents (i.e. adamantanes, benzenes, indanes, decalins, tetralins, biphenyls, diphenylmethanes and naphthalenes) and the pathways for the formation of these compounds were investigated to understand the major processes that result in their occurrence in groundwater. Comparisons of the polar compounds present in groundwater and equilibrated water phases were used to identify where the polar compounds have formed (groundwater or NAPL). Adamantanols, indanones, decalones and tetralones were present in both groundwater and equilibrated water phases, suggesting these compounds have formed and accumulated in the NAPL prior to partitioning into the groundwater. Assessment of possible biodegradation pathways for these compounds and the proportion of polar components in the soil profile suggested they are formed aerobically in the upper regions of the contaminated soil profile. Aromatic carboxylic acids were only identified in groundwater, suggesting they have formed from the biodegradation of water soluble aromatic compounds in this phase. Assessment of the mass spectra of polar UCMs from soil and groundwater associated with weathered diesel, and water equilibrated with weathered diesel showed similar mass spectral features that corresponded with cyclic carbon skeletons.

4.2 Introduction

Polar compounds formed from the degradation of petroleum have received little attention compared to their parent hydrocarbons, though a wide range of these polar compounds have been identified in surface (Ehrhardt and Burns, 1990; Ehrhardt and Burns, 1993), and subsurface environments (Langbehn and Steinhart, 1995; Angehrn *et al.*, 1998). Much of the literature reporting polar compounds formed from the biodegradation of petroleum has been focused on groundwater. Compound types identified largely contain aromatic carbon skeletons, and include benzoic, benzylsuccinic, naphthoic and tetrahydronaphthoic acids (Cozzarelli *et al.*, 1995; Gieg *et al.*, 1999; Beller, 2000; Ohlenbusch *et al.*, 2002; Namocatcat *et al.*, 2003). For the most part, the formation of these compounds is attributed to anaerobic processes, occurring in dissolved contaminant plumes of anoxic groundwater.

The occurrence and formation of polar compounds in soil is relatively less explored, though a range of polar compounds have been identified in contaminated soils (Langbehn and Steinhart, 1995), and contaminated soils that have undergone bioremediation (Angehrn *et al.*, 1998). Langbehn and Steinhart (1995) identified a range of polar compounds in soils affected by unknown petroleum contaminants. Polar compounds were suggested to have formed from the biodegradation of petroleum, and included cyclic carboxylic acids and aromatic alcohols and ketones. Polar compounds identified in ex-situ bioremediated soils associated with a range of petroleum contaminants (largely diesel and heating oil) include long chain alcohols, aldehydes, ketones, and carboxylic acids (Angehrn *et al.*, 1998).

Laboratory studies have also simulated the biodegradation of petroleum in soil, and analysed the polar compound types formed (Langbehn and Steinhart, 1995; Chaîneau *et al.*, 2005; Mao *et al.*, 2009). Identifications made from soil spiked with diesel and lubricating oil include a range of polar compound types, with the major carbon skeleton types of these compounds relating to the composition of the original petroleum. For example, higher abundances of branched polar compound types in soils spiked with

lubricating oils, and higher abundances of aromatic polar compound types in soils spiked with diesel (e.g. 1-hydroxy-2-naphthoic acid and 9-fluorenone-1-carboxylic acid) (Langbehn and Steinhart, 1995). Mao et al. (2009) also conducted a study simulating the biodegradation of petroleum in the vadose zone, analysing water leached organics from soil microcosms spiked with diesel. Compound types identified included aldehydes, ketones, diones, lactones, alcohols, phenols and carboxylic acids, as well as some high molecular weight oxygenated sulfur and nitrogen containing compounds (Mao *et al.*, 2009). Throughout the duration of these experiments it was noted that the molecular weight of the leached polar compounds increased, suggesting larger polar compounds were being formed with increased levels of biodegradation (Mao *et al.*, 2009).

4.3 Scope of study

The aim of this chapter is to investigate the occurrence of biodegradation products from weathered petroleum that are present in soil, groundwater and NAPL. A range of polar biodegradation products are identified (some identified for the first time) and their presence or absence in the different phases used to assess where they have formed. The types of compounds identified are also used to indicate the composition of polar UCMs associated with petroleum.

4.4 Experimental

4.4.1 Sample details

4.4.1.1 NAPL

The weathered diesel used for equilibration in Chapter 3 (Weathered diesel-1) was also used for equilibration in this chapter. Sampling details are described in section 3.4.1.

4.4.1.2 Soil

Soil samples associated with weathered diesel were collected from the same site as weathered diesel-1 by pushing an aluminium tube (50 mm diameter) directly into the ground. The soil profile (3.26 – 4.26 m below ground surface; bgs) was separated into 50 mm intervals and each sample stored separately in air-tight tins prior to sub-sampling and TPH analysis.

4.4.1.3 Groundwater

Groundwater samples (~500 mL) associated with the weathered diesel were collected from monitoring wells and previously constructed multiport samplers (bundles of 4.8 mm i.d. nylon tubing terminated with a screen of slotted aluminium tube, 200 mm long and 4.5 mm i.d.) at the same site as the soil and NAPL. Further details for the multiport sampler construction and installation can be found in Johnson *et al.* (1998).

4.4.2 Equilibration experiments

Equilibration experiments were performed using the procedure described in section 3.4.2.

4.4.3 Total petroleum hydrocarbons (TPH)

4.4.3.1 Soil

Soil samples (approx. 5 g) were extracted with diethyl ether/acetone (1:1 v/v, 5 mL) containing a surrogate standard (d8-naphthalene, 870 µg) by tumbling. The solvent was removed and dried using anhydrous sodium sulfate to provide a sample ready for GC-MS analysis. Calibration standards were prepared by spiking known amounts of diesel fuel into the extraction solvent (diethyl ether/acetone, 1:1 v/v, 5 mL) containing a surrogate standard (d8-naphthalene) and extracted using the same procedure used for the soil samples.

Quantifications of TPH were based on total ion chromatogram (TIC) response, normalised by the surrogate standard and compared to external TPH standards.

4.4.3.2 Water

TPH was extracted from groundwater samples (70 mL) using DCM (3 mL). Water samples were acidified (4.5 M HCl) and spiked with a surrogate standard d₈-naphthalene (5 µg) prior to the addition of DCM. After thorough mixing, the DCM layer was removed and dried using anhydrous sodium sulfate to provide a sample ready for GC-MS analysis. Dissolved organics (measured as TPH) were extracted from the water phase of weathered diesel equilibration experiments using the method described in section 3.4.3. Calibration standards were prepared by spiking known amounts of fresh diesel fuel into the water samples and extracted using the same procedure used for the water samples. Quantifications of TPH were based on total ion chromatogram (TIC) response, normalised to the d₈-naphthalene internal standard.

4.4.4 Compound class characterisation

Compound class characterisation of defunctionalised products was carried out using the procedure described in section 2.2.3.

4.4.5 Analysis by Gas Chromatography Mass Spectrometry (GC-MS)

GC-MS analysis was performed using an Agilent 5975 MSD interfaced with an Agilent 6890 gas chromatograph equipped with an Alltech AT-1 ms column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Samples were dissolved in dichloromethane and injected (2 µL) using a vaporising injector fitted to an autosampler. Helium was used as a carrier gas with a linear flow rate of 1 mL/min with the vaporising injector operated in splitless mode. The GC oven was temperature programmed from 35 °C (2 min), ramped to 310 °C (15 min) at 15 °C/min. The MSD was operated with an ionisation energy of 70 eV at 230 °C in scan mode (m/z 50-500).

4.4.5.1 Compound identification

Individual compound identifications were based on matching GC retention time and mass spectra characteristics of the compound of interest with authentic standards. Tentative identifications were based on mass spectra characteristics and GC retention behaviour. Library matches for some tentatively identified compounds were based on a NIST/EPA/NIH mass spectral library (version 2). Molecular ions were monitored for polar compounds of interest in almost all cases, particularly those where reference mass spectra could not be obtained (e.g. alkylnaphthoic acids). Daughter ions were monitored in instances where the molecular ions for the compounds of interest possessed a very low abundance (daughter ions produced chromatograms with the best response with the lowest background noise).

4.5 Results and discussion

Water, soil and NAPL samples recovered from the environment were used to study polar compounds from the biodegradation of weathered diesel.

4.5.1.1 Groundwater TPH

Groundwater TPH associated with weathered diesel that was extracted from a range of samples (from the same site) typically consisted of a large UCM (Fig. 4.1). UCM profiles varied significantly, as did the number of resolved peaks (including aromatic and polar compounds).

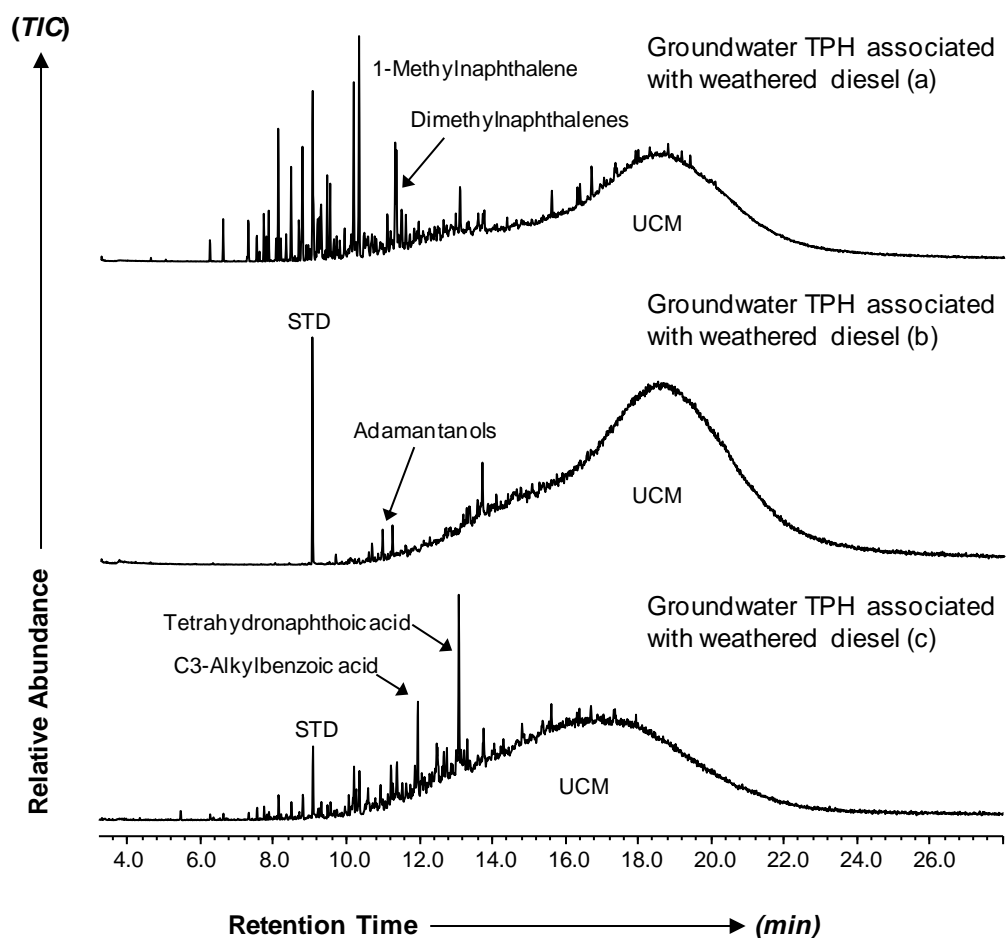


Figure 4.1 Total ion chromatograms (TICs) for groundwater TPH associated with a-c) weathered diesel from the same site. Chromatograms background subtracted to remove column bleed. STD: surrogate standard, UCM: unresolved complex mixture.

4.5.1.2 Weathered diesel equilibrated water phase

Water equilibrated with weathered diesel (Fig. 4.2) was analysed to investigate individual polar compounds that accumulate in weathered petroleum. TPH from water equilibrated with the weathered diesel primarily consists of large UCM, though several resolved peaks are also present.

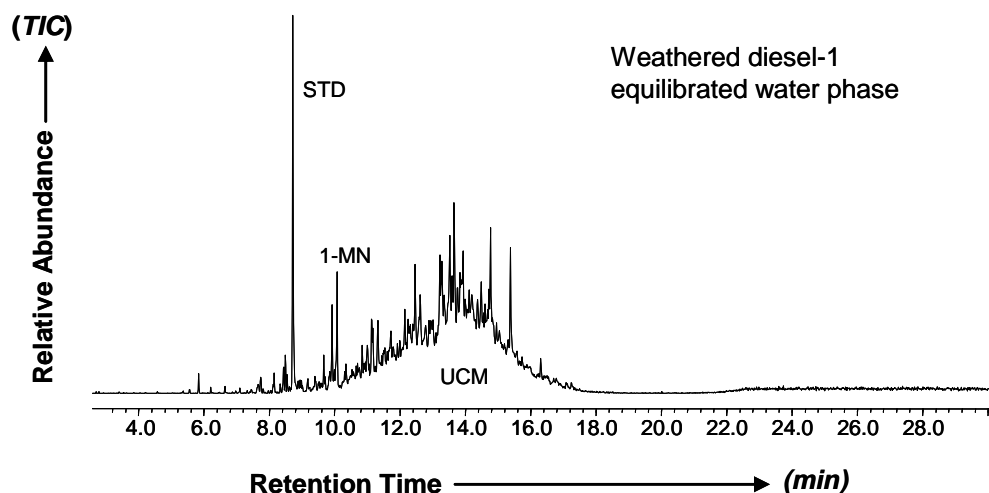


Figure 4.2 Total ion chromatogram (TIC) for weathered diesel-1 equilibrated water phase. Chromatograms background subtracted to remove column bleed. ISTD: internal standard, UCM: unresolved complex mixture

4.5.1.3 Soil profile TPH

A soil profile associated with weathered diesel was analysed for TPH to develop an understanding of areas in the soil profile where polar compounds may form from biodegradation processes (Fig. 4.3). TPH from the top of the soil profile (Fig. 4.3a) did not contain pristane and phytane and were assessed as being significantly biodegraded compared to TPH lower in the soil profile (Fig. 4.3b). The increased TPH concentration measured in the lower half of the soil profile (maximising at 3.86 m bgs) likely corresponds with a large amount of NAPL present above the water table which resides at approximately 4.01 to 4.06 m bgs (Fig. 4.4).

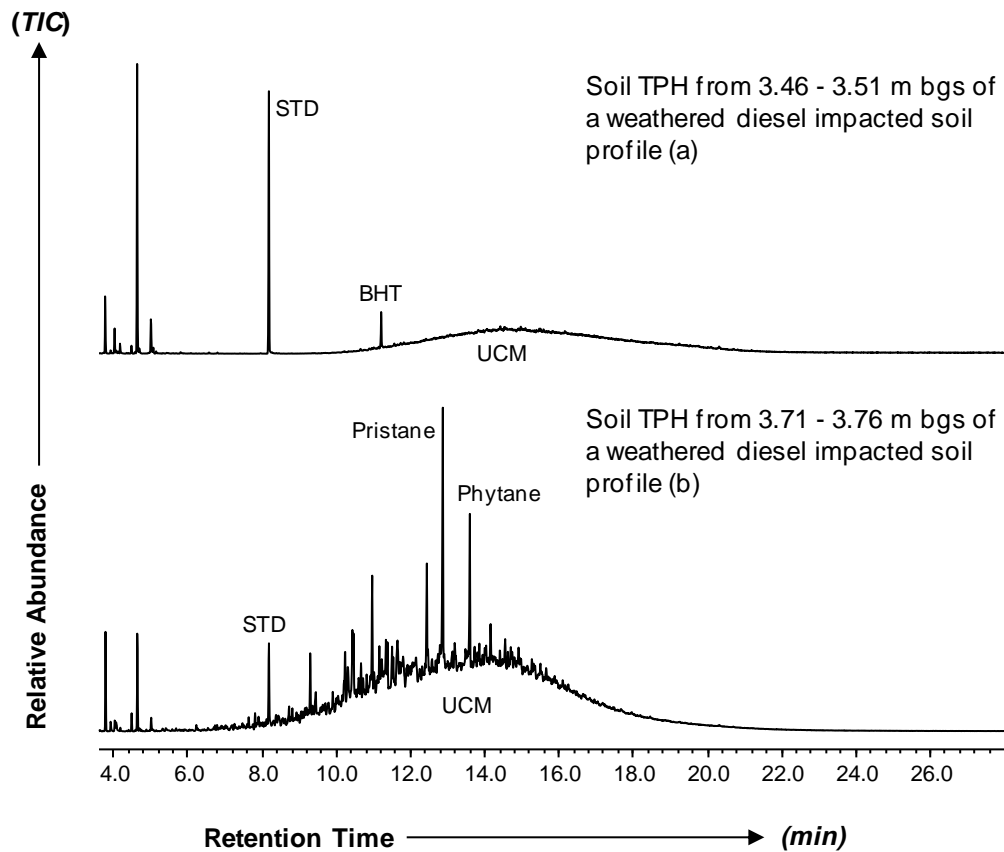


Figure 4.3 Total ion chromatograms (TICs) for soil TPH associated with weathered diesel at a) 3.46-3.51 m and b) 3.71-3.76 m below ground surface (bgs). Chromatograms were background subtracted to remove column bleed. STD: surrogate standard, BHT: butylated hydroxytoluene (analytical artefact), UCM: unresolved complex mixture.

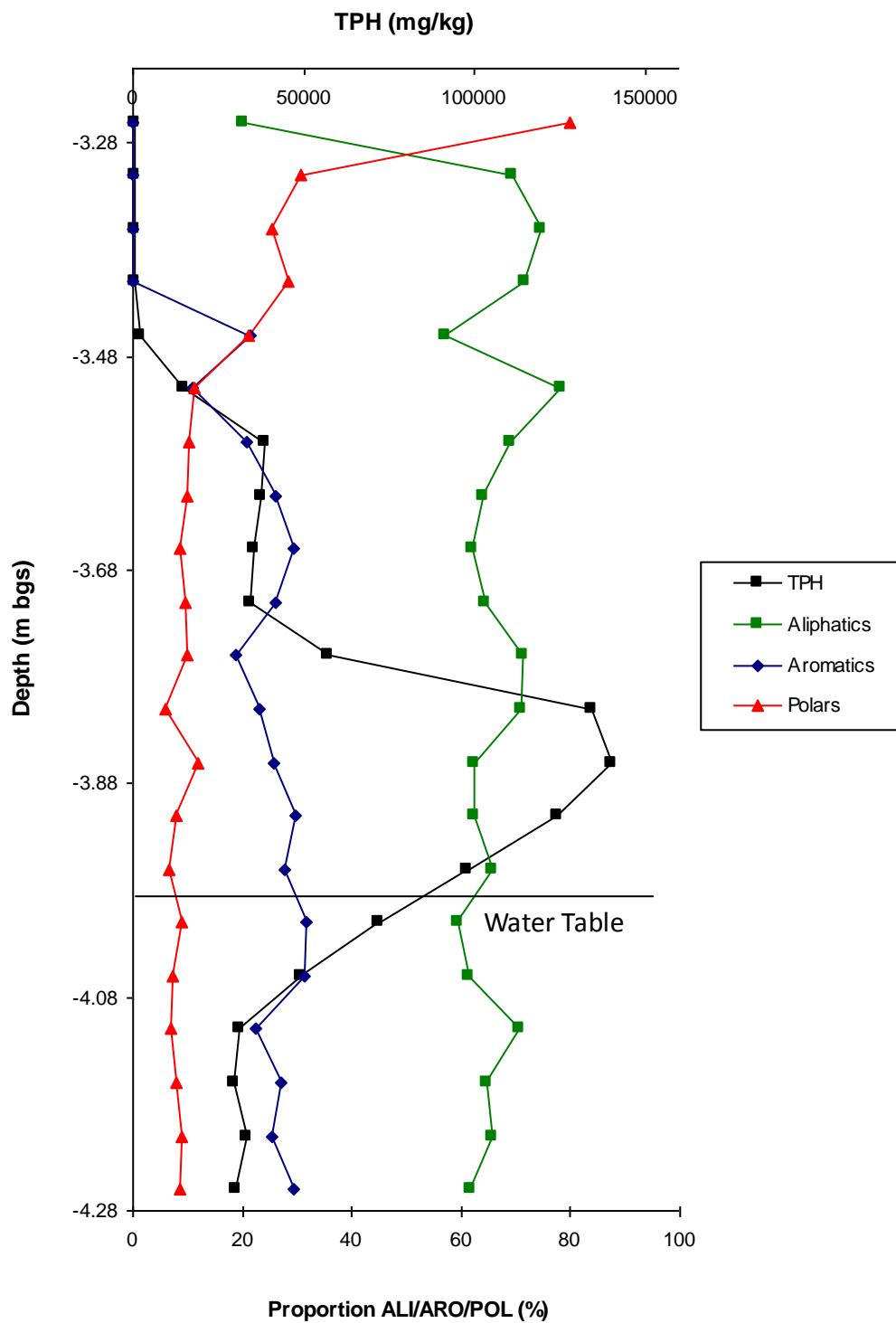


Figure 4.4 TPH of a soil profile impacted with weathered diesel and the proportion of aliphatic, aromatic and polar components. ALI: Aliphatic fraction, ARO: Aromatic fraction, POL: polar fraction.

Compound class characterisation of TPH in the soil profile was carried out to assess the proportion of aliphatic, aromatic and polar components present (Fig. 4.4). Differences in these proportions may provide evidence for the formation of polar compounds in a specific region of the soil profile. Aliphatic components represent the highest proportion (average of 66 %) of the TPH throughout the soil profile, except for the shallowest depth measured where they represent the lowest proportion of the TPH (20 %). Aromatic components initially represent a very low proportion of the TPH at the shallowest depths measured, increasing to a relatively constant proportion with increasing depth (average of 27 % from 3.61 to 4.26 m bgs). Polar components represent the highest proportion of the TPH at the shallowest depth measured (80 %), gradually decreasing before remaining relatively constant below a depth of 3.51 m bgs (average of 9 %). These differences in the proportion of aliphatic, aromatic and polar compounds with depth suggest the proportion of polar components is largest at the top of the soil profile where biodegradation appears to be the greatest, and corresponds with a general decrease in the proportion of aliphatic and aromatic components. The larger proportion of polar compounds at the top of the soil profile suggests the formation of polar compounds may be a dominant process in this region. Evidence for the increased biodegradation of petroleum in this region is suggested by the removal of pristane and phytane from the TPH, and possibly attributed to aerobic conditions that take place in the uppermost regions of the soil profile (Davis *et al.*, 1998). Mixing of the polar compounds formed at the top of the soil profile into the main NAPL body may occur through fluctuations in the water table, resulting in the continuous formation and accumulation of polar compounds in the NAPL.

4.5.2 Polar compounds identified

Polar compound types identified in environmental samples included alcohols, phenols, aldehydes, ketones, sulfoxides and carboxylic acids (Table 4.1). Carbon skeleton types for these compounds were varied, and related to a range of cyclic hydrocarbons and heterocyclic compounds from petroleum.

Table 4.1 Polar compounds identified in water associated with weathered diesel.
GW: Groundwater, EQ: Equilibrated water phase.

Compound type	Identification	Sample type	Ions monitored (m/z)
Adamantanols			
1-Adamantanol	Authentic Standard	GW, EQ	152, 95
C1-Alkyladamantanes	Tentative	GW, EQ	166, 109, 95
C2-Alkyladamantanes	Tentative	GW, EQ	166, 109, 95
Bicyclic alcohols			
4-methylbicyclo[2,2,2]octanol	Tentative	GW	140, 111, 70
Phenols			
3,4,5-Trimethylphenol	Authentic Standard	GW	136, 121, 91
C3-Alkylphenols	Tentative	GW	136, 91
Indanols			
6-Methyl-4-indanol	Tentative	GD	148, 133
Indanones			
1-Indanone	Authentic Standard	Soil	132, 117
2-Methyl-1-indanone	Authentic Standard	GW, EQ	146, 131, 117
Methylindanones	Tentative	GW, EQ	146, 117
Dimethylindanones	Tentative	GW, EQ	160, 117
Decalones			
Decalone	Tentative	GW, EQ	152, 108, 55,
Methyldecalone	Tentative	GW, EQ	166, 95, 55,
Tetralones			
1-Tetralone	Authentic Standard	Soil	146, 118, 90
2-Methyl-1-tetralone	Authentic Standard	GW, EQ, soil	160, 118, 90
Methyltetralones (<i>benzylic methyl group</i>)	Tentative	GW, EQ	160, 132, 104
Methyltetralones (<i>cyclic methyl group</i>)	Tentative	GW, EQ	160, 118
Sulfoxides			
8-Methyl-7-thiabicyclodecane sulfoxide	Tentative	EQ	186, 168, 95
Ketones			
1-Thiaacenaphthen-2-one	Tentative	EQ	186, 158, 159
Benzoic acids			
2,4,6-Trimethylbenzoic acid	Authentic Standard	GW	164, 146, 119

C3-alkylbenzoic acids	Tentative	GW	164, 146, 119
C3-alkylbenzoic acid	Tentative	GW	178, 163
<i>Biphenylacetic acids</i>			
Biphenylacetic acid	Tentative	GW	212, 167
Methylbiphenylacetic acids	Tentative	GW	228
<i>Diphenylethanoic acids</i>			
Diphenylethanoic acid	Tentative	GW	212, 167
Methyldiphenylethanoic acids	Tentative	GW	228
<i>Naphthaleneacetic acids</i>			
Naphthaleneacetic acids	Tentative	GW	186, 141, 115
Methylnaphthaleneacetic acids	Tentative	GW	200, 141, 115
C2-Alkyl naphthaleneacetic acids	Tentative	GW	214, 141, 115
<i>Naphthioic acids</i>			
Methylnaphthioic acids	Tentative	GW	186, 141, 115
C2-Alkyl naphthioic acids	Tentative	GW	200, 141, 115
C3-Alkyl naphthioic acids	Tentative	GW	214, 141, 115
<i>Indanoic acids</i>			
Indanoic acid	Tentative	GW	162, 117
<i>Tetrahydronaphthoic acids</i>			
5,6,7,8-Tetrahydro-1-naphthoic acid	Tentative	GW GW	176, 158, 131, 115

4.5.2.1 Alcohols

Cyclic alcohols were identified included adamantanols, phenols and indanols that are likely formed from the degradation of petroleum.

4.5.2.1.1 Adamantanols

Adamantanols were identified in groundwater associated with weathered diesel (Fig. 4.5a) and water equilibrated with weathered diesel (Fig. 4.5b) with very similar distribution. Adamantanols identified included 1-adamantanol (Fig. 4.6a; identified using an authentic standard) and

tentatively identified methyl- (Fig. 4.6b) and dimethyladamantanols (Fig. 4.6c). The mass spectra for dimethyladamantanols (Fig. 4.6d,e) were similar to C2-alkyldecalones, though retention time characteristics suggest these compounds are dimethyladamantanols. To the authors knowledge, adamantanol has not been previously identified in groundwater associated with weathered petroleum.

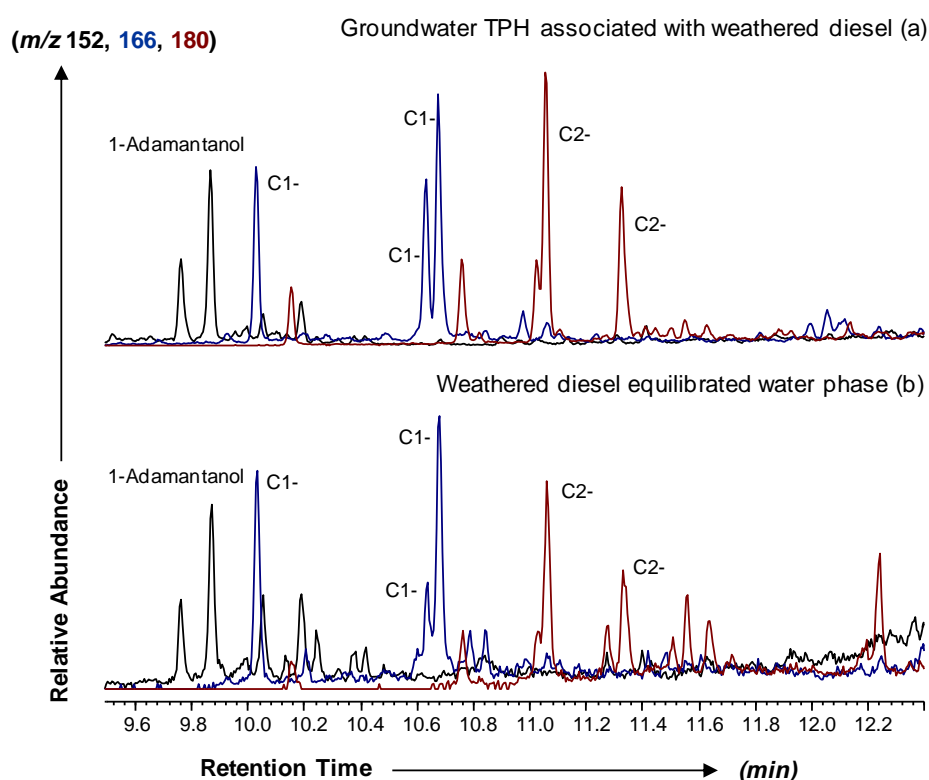


Figure 4.5 Partial ion chromatograms (m/z 152, 166 and 180) showing adamantanol, and C1- and C2-alkyladamantanols in a) groundwater associated with weathered diesel and b) the equilibrated water phase for weathered diesel.

Adamantanols present in groundwater associated with weathered diesel and water equilibrated with weathered diesel are likely formed from the aerobic biodegradation of adamantanes. Adamantanes are common constituents of petroleum (Mair *et al.*, 1959; Petrov *et al.*, 1974) that have been suggested to be affected by biodegradation after most hydrocarbons have been removed (Grice *et al.*, 2000). Laboratory studies documenting aerobic biodegradation

pathways of adamantanes show the position of oxidation is highly selective, producing exclusively tertiary alcohols (i.e. hydroxylation at the 1- and 1,3-positions;(Slepenkin *et al.*, 1993; Bagrii, 1995). The presence of only 1-adamantanol (and absence of 2-adamantanol) and only three (tentatively identified) methyladamantanol isomers suggests hydroxylation has been selective to the tertiary position, as only three methyl-1-admantanol configurations are possible. The selectivity of the adamantanol biodegradation products suggests they have formed through previously documented aerobic processes.

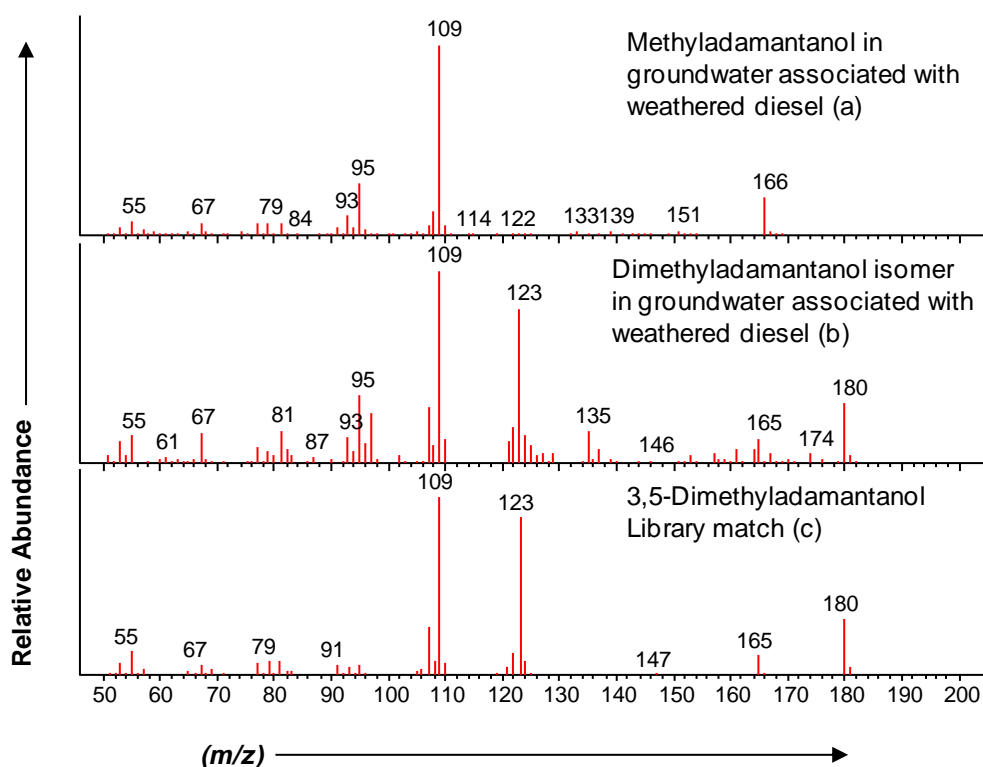


Figure 4.6 Mass spectra for a) a tentatively identified methyladamantanol, b) a tentatively identified dimethyladamantanol and c) 3,5-dimethyladamantanol library spectrum.

No evidence for the formation of adamantanols through other biodegradation processes could be found in the samples studied (2-adamantanol was not present), though the degradation of adamantane via a 2-admantanol intermediate has been reported (White *et al.*, 1984). The absence of 2-

adamantanol in the present study could therefore be a result of further oxidation through a series of transformation products (Fig. 4.7; Wei *et al.* 2007). None of these transformation products could be identified in the present study, suggesting the most likely pathway for adamantane biodegradation is the selective hydroxylation of tertiary carbon atoms (1- and 1,3- positions) by aerobic biodegradation.

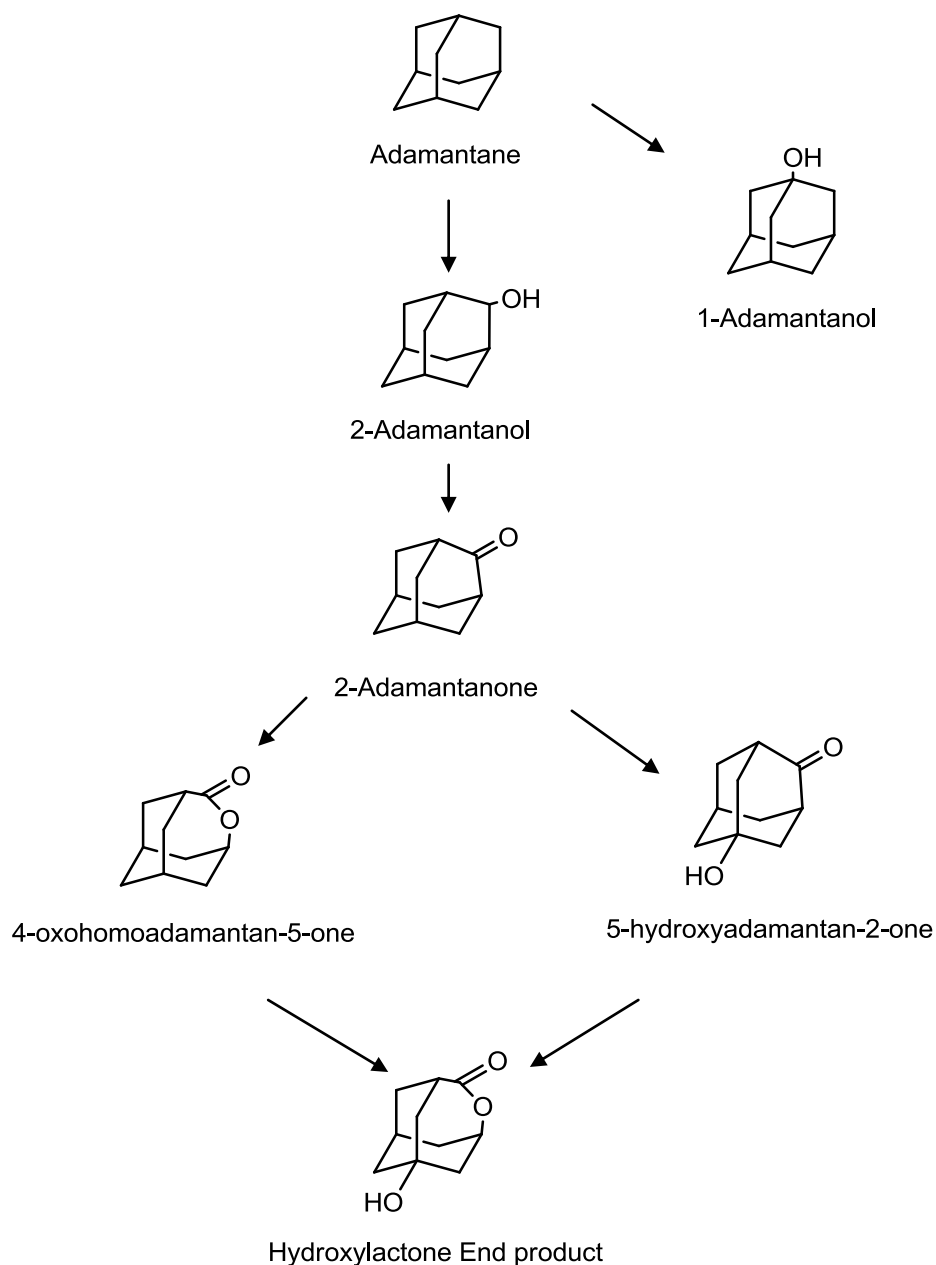


Figure 4.7 Proposed transformation pathways for adamantane. Modified from Selifonov (1992) and Wei *et al.* (2007).

Similar distributions of alkyladamantanols in the groundwater associated with weathered diesel and water equilibrated with weathered diesel suggests they have accumulated in the NAPL. Therefore, the presence of adamantanol in groundwater is likely a result of their partitioning from NAPL, rather than their formation in groundwater. This is expected, considering adamantanes are aliphatic compounds that are not water soluble and not likely to be present in groundwater to undergo biodegradation.

4.5.2.1.2 Bicyclic alcohols

A bicyclic alcohol was tentatively identified in groundwater associated with weathered diesel (Fig. 4.8). The presence of this compound (4-methylbicyclo[2,2,2]octanol; Fig. 4.9) in groundwater associated with petroleum contamination has not been previously reported, to the knowledge of the author.

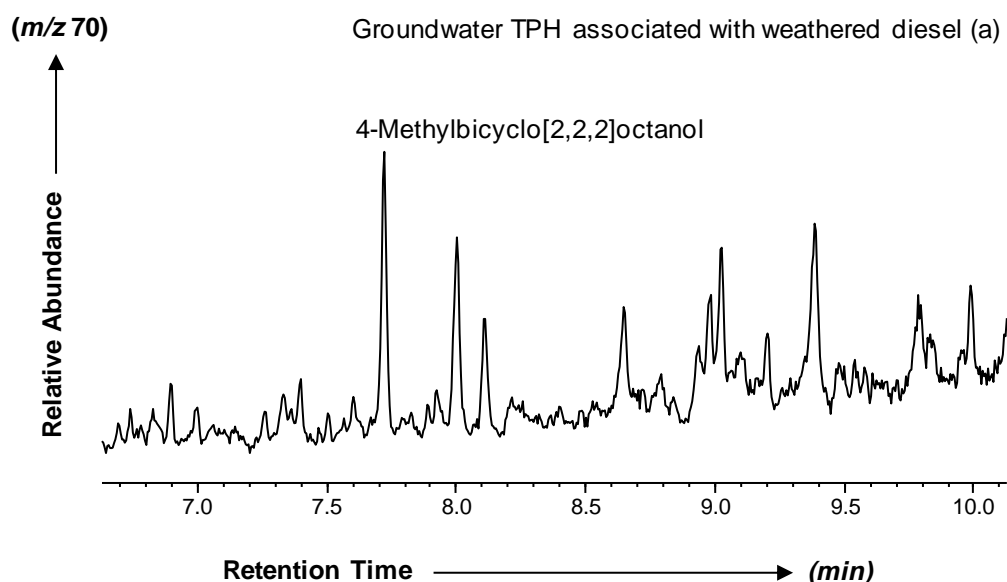


Figure 4.8 Partial ion chromatograms (m/z 70) showing 4-methylbicyclo[2,2,2]octanol in groundwater associated with weathered diesel.

The occurrence of the bicyclic alcohol in water samples associated with weathered petroleum is likely a result of its formation from the oxidation of a

hydrocarbon containing the same carbon skeleton, specifically 4-methylbicyclo[2,2,2]octane (Fig. 4.10). This putative parent compound is not water soluble and the compound is therefore not likely to have formed in the groundwater. Only one isomer of bicyclic alcohol could be identified (assuming the isomers do not co-elute), which suggests oxidation has occurred selectively at the tertiary position similar to the adamantanol, and therefore via aerobic oxidation.

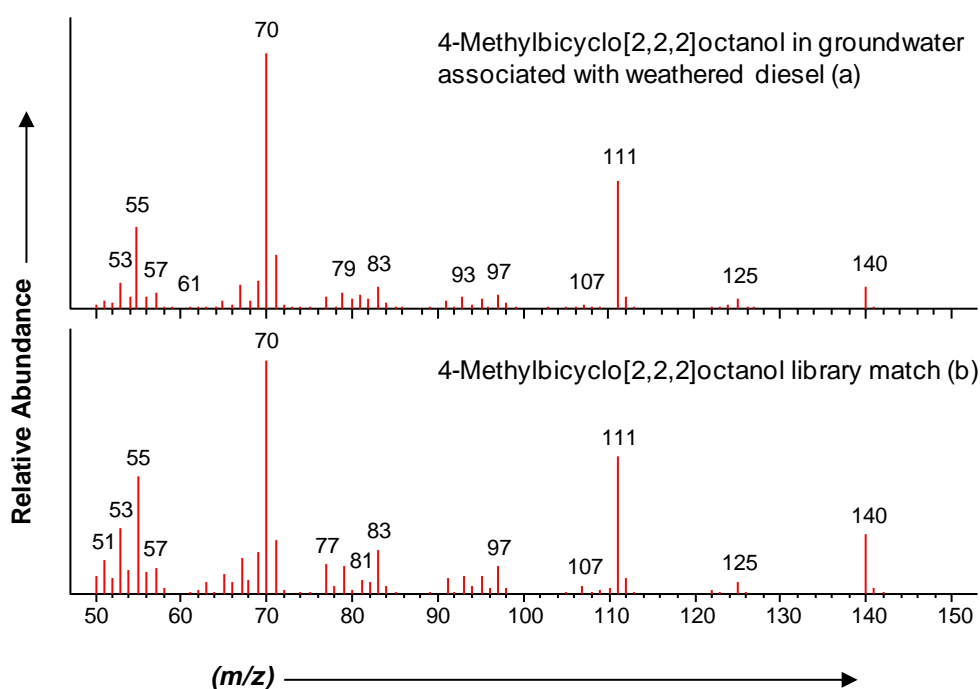


Figure 4.9 Mass spectrum for a) tentatively identified 4-methylbicyclo[2,2,2]octanol and b) the library spectrum for 4-methylbicyclo[2,2,2]octanol.



Figure 4.10 Proposed transformation pathway for the formation of 4-methylbicyclo[2,2,2]octanol.

4.5.2.1.3 Alkylphenols

Alkylphenols were identified in groundwater associated with weathered diesel (Fig. 4.11). These alkylphenols primarily included C3-alkylphenols, with 3,4,5-trimethylphenol identified (using an authentic standard) as well as three C3-alkylphenol isomers identified using a known elution order (Bastow *et al.*, 2003). The mass spectrum for 2,3,6-trimethylphenol identified by elution order is shown in Figure 4.12. Mass spectra included a range of interfering ions that are not present in the library match. This shows the difficulties associated with the identification of even relatively well resolved components in TPH extracts containing a large UCM.

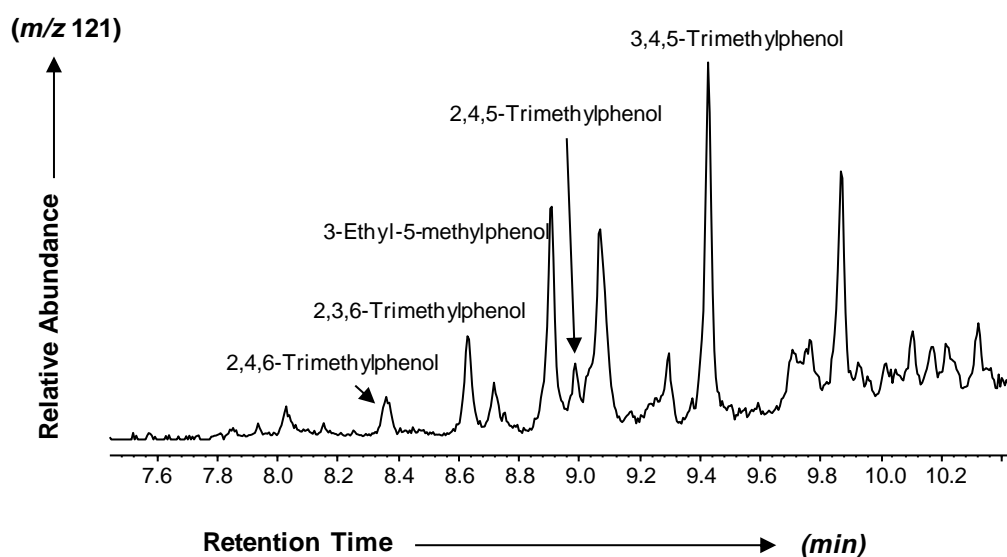


Figure 4.11 Partial ion chromatograms (m/z 121) showing C3-alkylphenols in groundwater associated with weathered diesel.

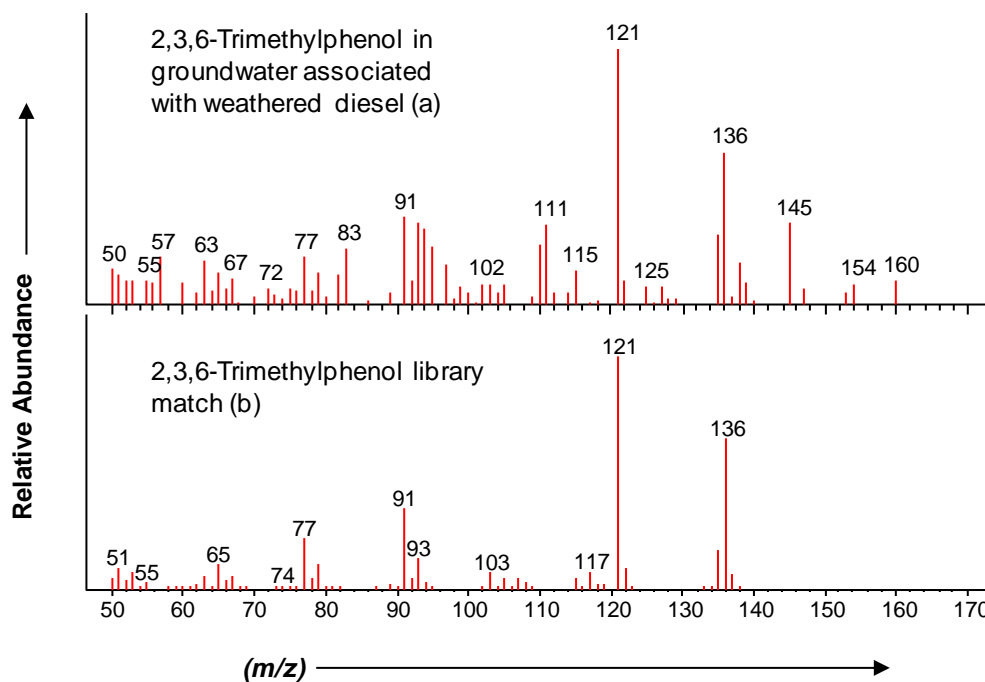


Figure 4.12 Mass spectrum for a) 2,3,6- trimethylphenol in groundwater associated with weathered diesel and b) the library spectrum for 2,3,6-trimethylphenol.

Alkylphenols in groundwater are reported biodegradation products of alkylbenzenes (Fig. 4.13) and suggested to form through anaerobic and aerobic processes. Aerobic biodegradation is suggested to occur via hydroxylation at different positions of the benzene ring (Gibson *et al.*, 1970; Shields *et al.*, 1989; Whited and Gibson, 1991; Fritsche and Hofrichter, 1999). Alternatively, anaerobic biodegradation of benzene is suggested to occur through a phenol intermediate (Foght, 2008), and the anaerobic biodegradation of propylbenzene shown to form propylphenols (Eriksson *et al.*, 2005).

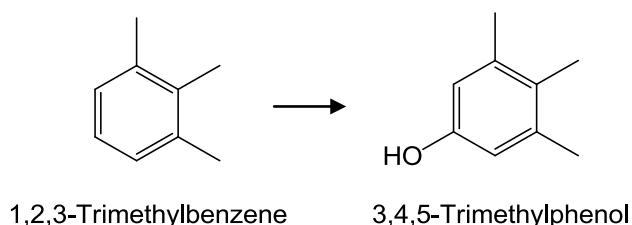


Figure 4.13 Proposed pathway for the formation of 3,4,5-trimethylphenol from 1,2,3-trimethylbenzene.

As C3-alkylbenzenes are expected parent compounds to the C3-alkylphenols, the comparison of the C3-alkylbenzenes in groundwater TPH associated with weathered diesel with those present in a weathered diesel recovered from the same site may show differences that help relate C3-alkylphenols to their parent compounds. Inspection of the C3-alkylbenzenes present in the groundwater (Fig. 4.14) show the trimethylbenzenes have been severely depleted due to biodegradation processes. The trimethylbenzene reported to be the most biodegradation resistant has also been severely depleted in the groundwater sample (1,2,3-trimethylbenzene; George *et al.* 2002), which is a likely parent compound to the dominant alkylphenol isomer (3,4,5-trimethylphenol) present in the groundwater. The dominance of 3,4,5-trimethylphenol in groundwater therefore reflects the extent of biodegradation the weathered diesel has experienced, with the likely biodegradation product of the most resistant trimethylbenzene isomer still remaining.

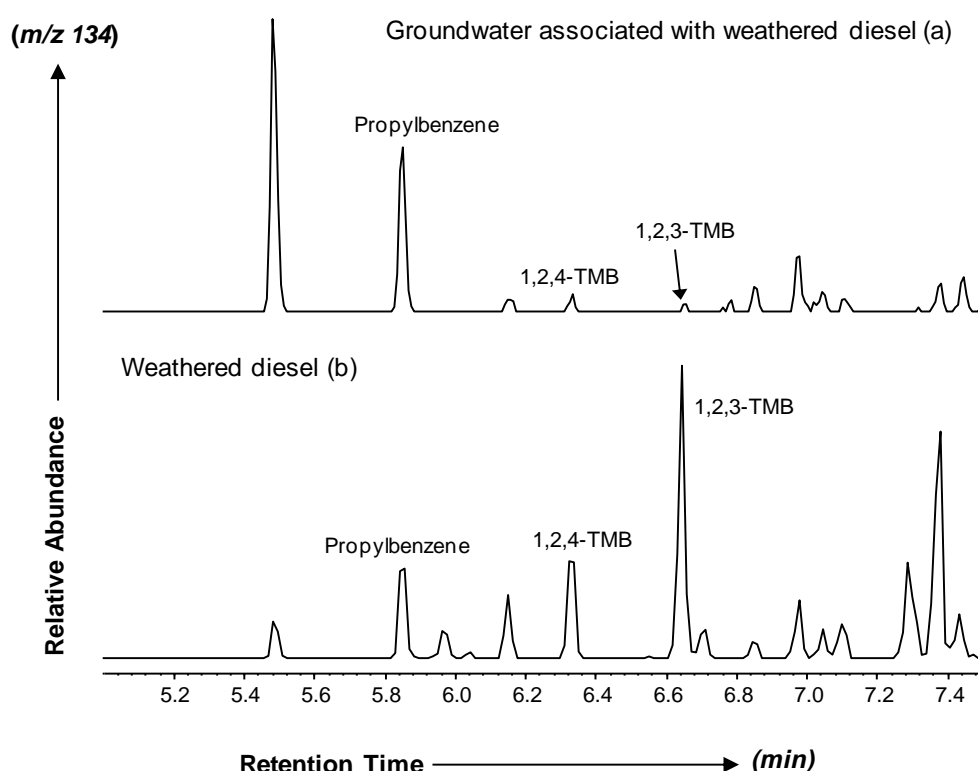


Figure 4.14 Partial ion chromatograms (134 m/z) showing trimethylbenzenes present in a) groundwater associated with weathered diesel and b) a weathered diesel. TMB: trimethylbenzene.

4.5.2.1.4 Indanols

A methylindanol was tentatively identified in groundwater associated with weathered diesel (Fig. 4.15). The mass spectrum of the compound closely matched the mass spectrum of 6-methyl-4-indanol (Fig. 4.16), suggesting the compound is an indanol with the hydroxyl group attached to the aromatic ring.

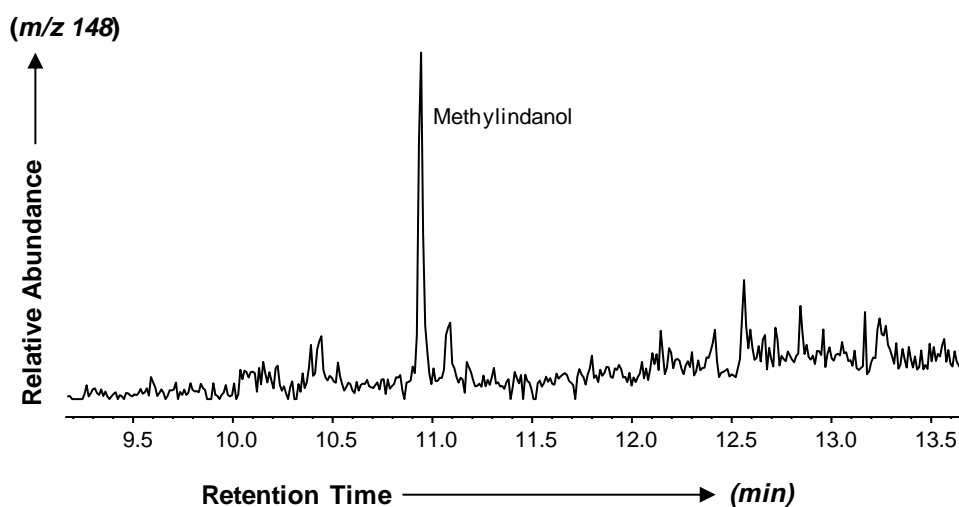


Figure 4.15 Partial ion chromatogram (m/z 148) showing the tentatively identified methylindanol in groundwater associated with weathered diesel.

Indanols are likely formed from the biodegradation of indanes (Fig. 4.17) in the groundwater, as they were not identified in the water equilibrated with weathered diesel. The biodegradation of indanes has been reported aerobically through selective hydroxylation at the benzylic carbon atom to produce a 1-indanol intermediate (Brand *et al.*, 1992; Gibson *et al.*, 1995; Lie *et al.*, 2009) or simultaneous hydroxylation at the 4- and 5-positions to form 5-indanol (Kim *et al.*, 2010). A similar process may account for the formation of the methylindanol isomer with the hydroxyl group attached to the aromatic ring in groundwater associated with weathered diesel.

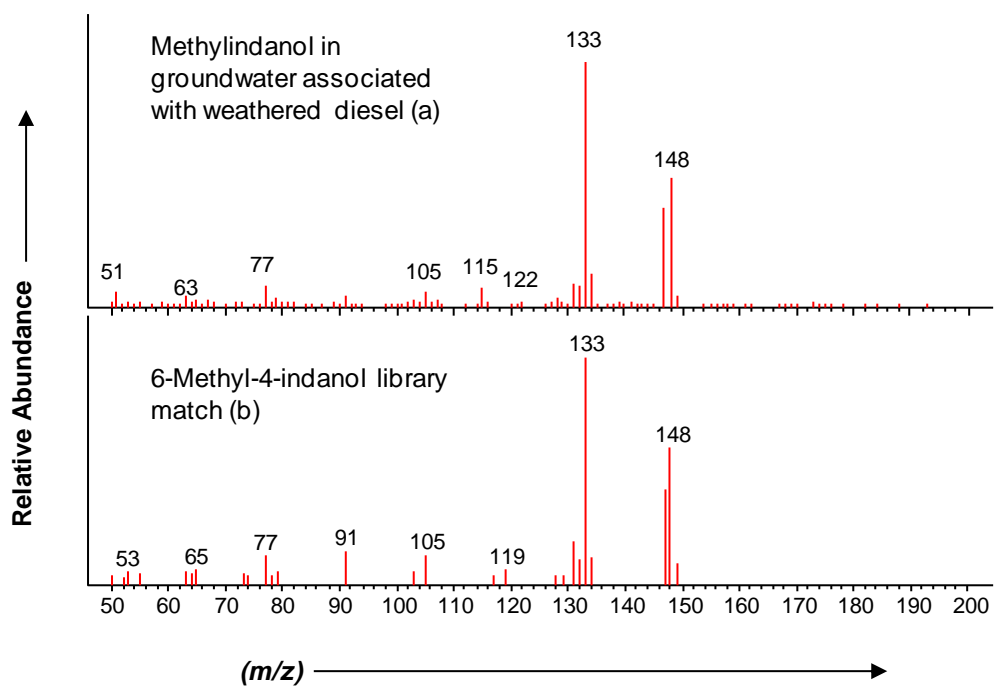


Figure 4.16 Mass spectrum of a) tentatively identified methylindanol in groundwater associated with weathered diesel and b) the library spectrum for 6-methyl-4-indanol.

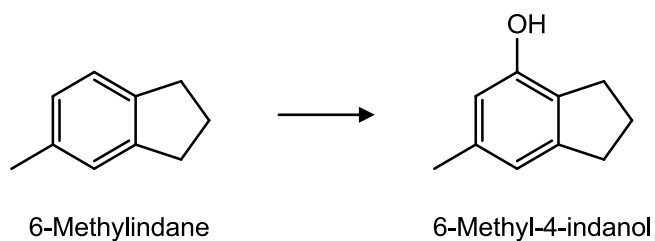


Figure 4.17 Proposed pathway for the formation of 6-methyl-4-indanol from 6-methylindane.

4.5.2.2 Ketones

Polar compounds containing ketone functional groups were identified in some of the samples. This included indanones, decalones and tetralones.

4.5.2.2.1 Indanones

Indanones were identified in soil and groundwater associated with weathered diesel, and water equilibrated with weathered diesel. This included the identification of 2-methyl-1-indanone (using an authentic standard) and the tentative identification of a range of methylated isomers (Figs. 4.18,19). Unsubstituted indanones were not present in groundwater TPH and equilibrated water phases, though a range of dimethylindanones were tentatively identified (Figs. 4.19, 21). The occurrence of these alkylindanones in groundwater associated with weathered petroleum has not been previously reported, to the knowledge of the author.

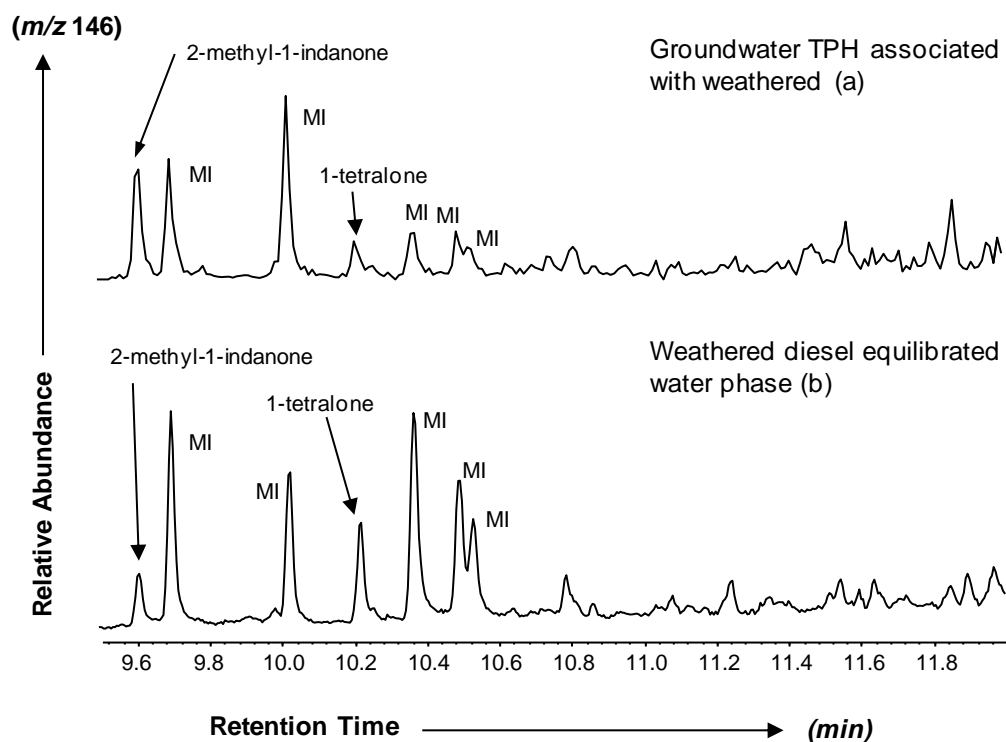


Figure 4.18 Partial ion chromatogram (m/z 146) showing 1-tetralone and methylindanones in a) groundwater associated with weathered diesel, and b) weathered diesel equilibrated water phase. MI: Methylindanone.

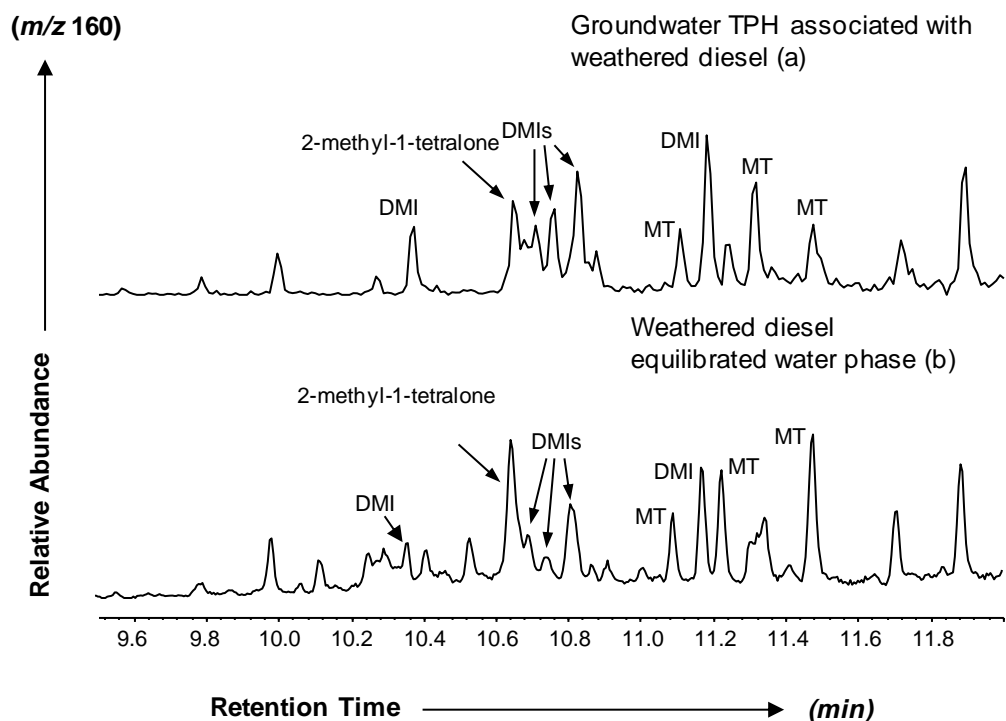


Figure 4.19 Partial ion chromatogram (m/z 160) showing a) methyltetralones and dimethylindanones present in a) groundwater associated with weathered diesel and b) equilibrated water phase. MI: Methylindanone. DMI: Dimethylindanone, MT: Methyltetralin.

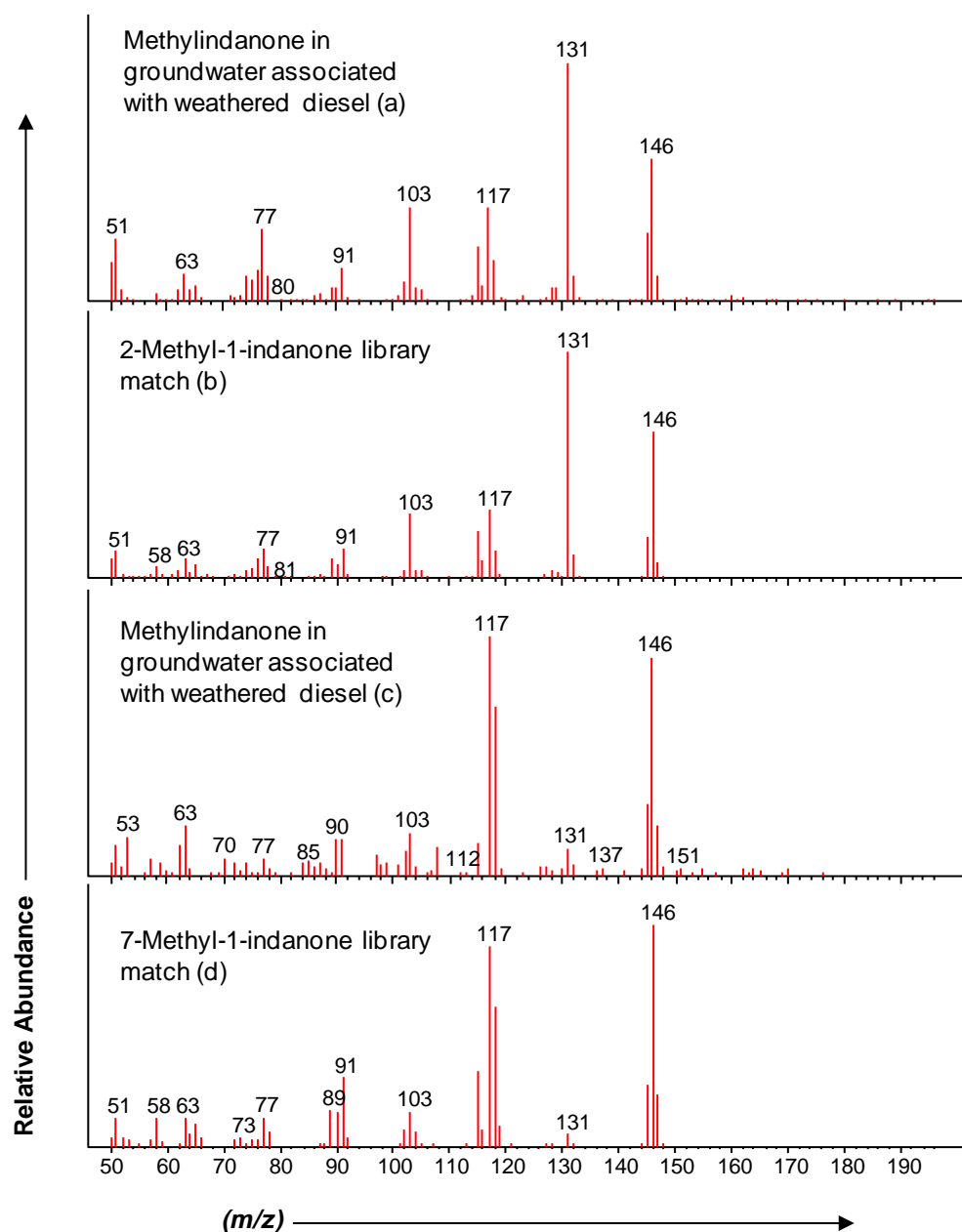


Figure 4.20 Mass spectra for a) tentatively identified methylindanone isomer (methyl group attached to the cyclic ring), b) library spectrum for 2-methyl-1-indanone, c) a tentatively identified methylindanone isomer (methyl group attached to the aromatic ring) and d) library spectrum for 7-methyl-1-indanone.

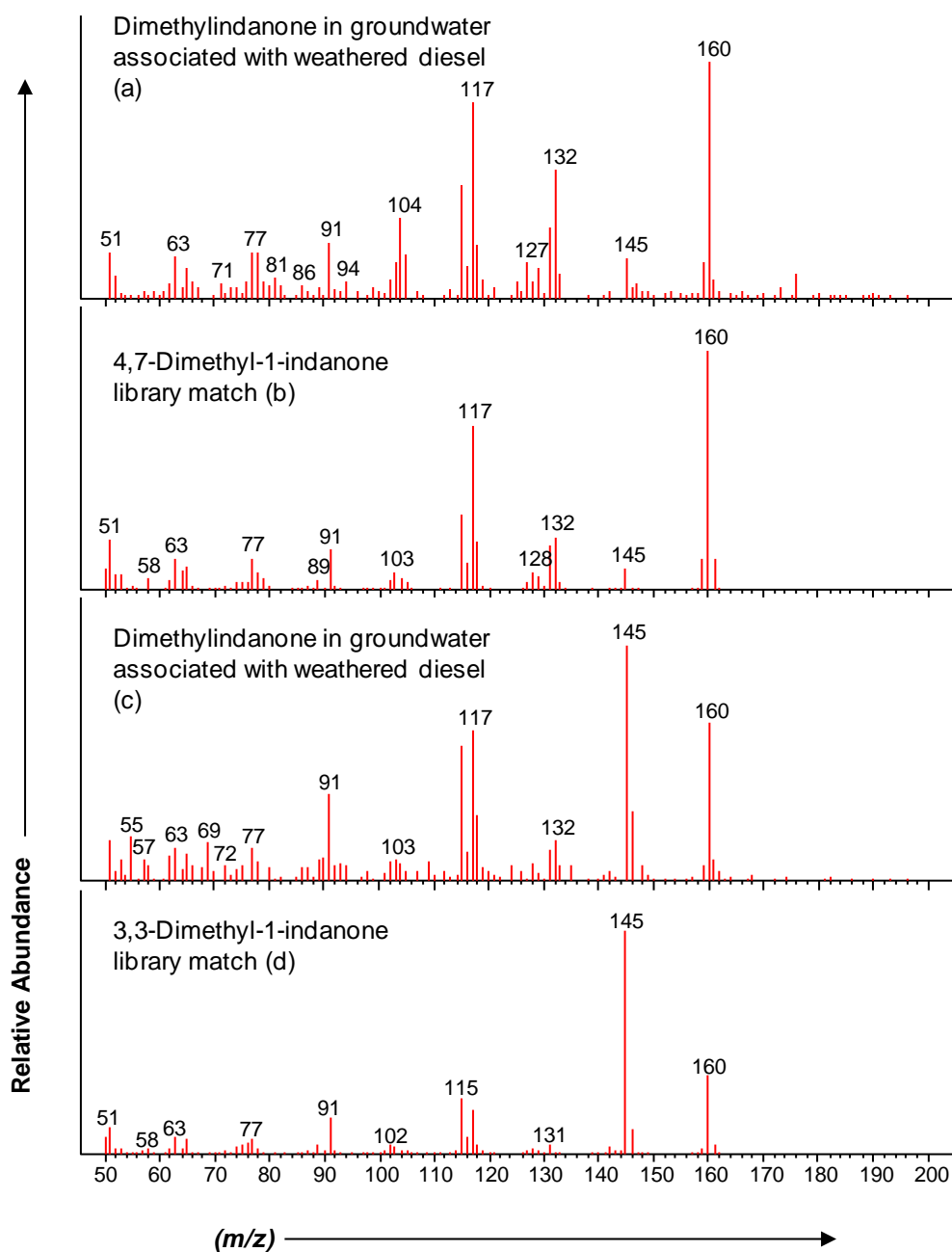


Figure 4.21 Mass spectra for a) a tentatively identified dimethylindanone isomer (methyl groups attached to the aromatic ring), b) library spectrum for a 4,7-dimethyl-1-indanone, c) a tentatively identified dimethylindanone isomer (methyl groups attached to the cyclic ring) and d) library spectrum for 3,3-dimethyl-1-indanone.

1-Indanone was identified (using an authentic standard) in only soil associated with weathered diesel (Fig. 4.22). 1-Indanone was the only unsubstituted indanone isomer identified, with the 2-indanone isomer not present in all soil TPH extracts analysed.

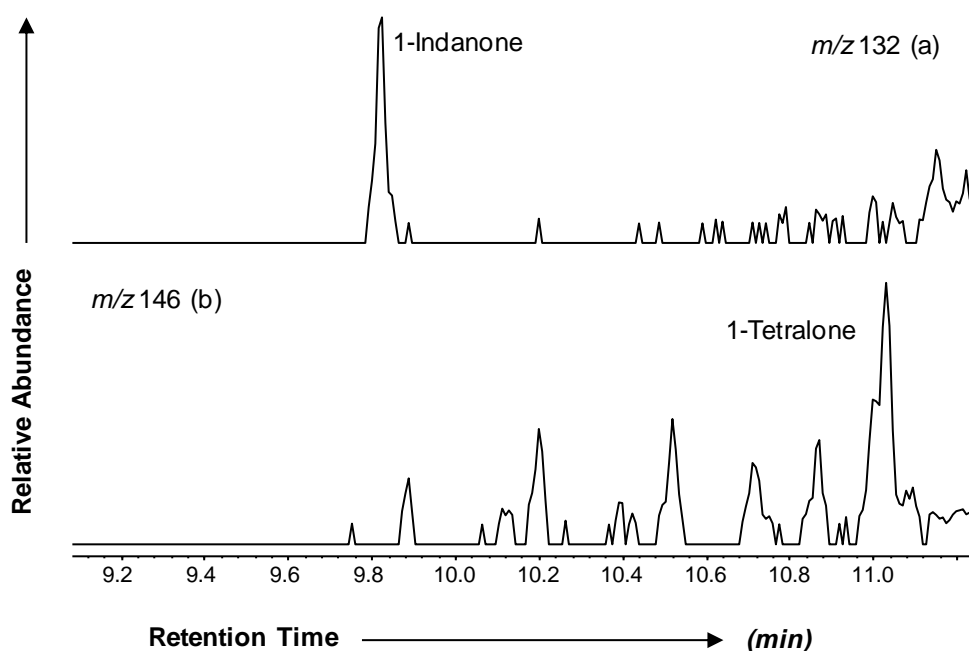


Figure 4.22 Partial ion chromatograms (m/z 132 and 146) showing methyl a) 1-indanone and b) 1-tetralone in soil associated with weathered diesel.

Previous field studies have documented the presence of 1-Indanone in anoxic groundwater plumes associated with coal tar contamination (Zamfirescu and Grathwohl, 2001; Mundt and Hollender, 2005). Laboratory studies have reported the formation of 1-indanone from the aerobic biodegradation of indane and indene, with biodegradation being initiated by selective hydroxylation of a benzylic carbon atom (i.e. the 1-position) to form 1-indanone only; Fig. 4.23) (Brand *et al.*, 1992; Gibson *et al.*, 1995; Mundt *et al.*, 2003; Lie *et al.*, 2009). The detection of six methylindanone isomers also suggests that the hydroxylation of these compounds has been selective at the 1- position, as only six methyl-1-indanone configurations are possible.

The formation of indanones through anaerobic biodegradation processes has not been documented, with the anaerobic biodegradation of indane shown to form an indanoic acid as a co-metabolite (Safinowski *et al.*, 2006) (Fig. 4.23). The lack of literature documenting indanones as anaerobic biodegradation products and the identification of exclusively 1-indanone isomers provides evidence that the indanones in the present study have formed from aerobic processes.

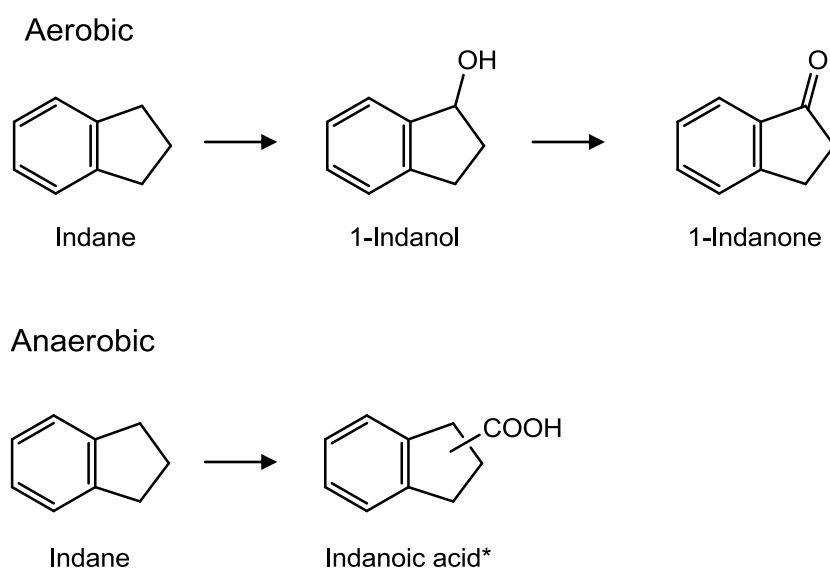


Figure 4.23 Pathways for the aerobic and anaerobic biodegradation of indane.
*Position of carboxylic group not specified.

The occurrence of indanones in both groundwater and water equilibrated with weathered diesel (Figs. 4.18-19) suggests they have accumulated in the NAPL before partitioning to groundwater. Though a similar range of isomers was identified in the groundwater and the equilibrated water phase, some differences exist in the distribution of the compounds. These differences are likely a result of alkylindanones undergoing further degradation in the groundwater after partitioning from the weathered diesel.

4.5.2.2.2 Decalones

Decalones were identified in groundwater associated with weathered diesel (Fig. 4.24). 2-Decalone and a methyldecalone isomer were tentatively identified based on mass spectra characteristics (Fig. 4.25). 1-Decalone was not present based on comparison with an authentic standard. The occurrence of these alkyldecalones in groundwater associated with weathered diesel has not been previously reported to the knowledge of the author.

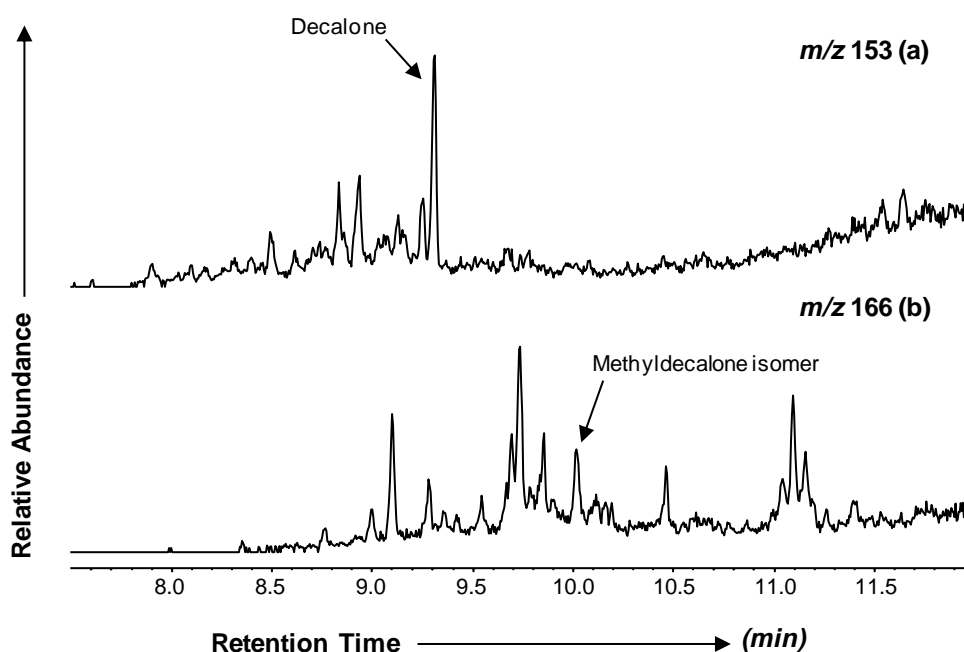


Figure 4.24 Partial ion chromatograms (m/z 152 and 166) showing a) a decalone and b) a methyldecalone in groundwater associated with weathered diesel.

The decalones identified in the samples are likely formed from the biodegradation of decalins. Decalones were not identified in water equilibrated with weathered diesel, though they are not likely to have formed in the groundwater due to decalins possessing negligible water solubilities. Therefore, as suggested for indanones and alkyladamantanols, decalones have likely formed in the soil profile before accumulating in the weathered diesel and partition into groundwater.

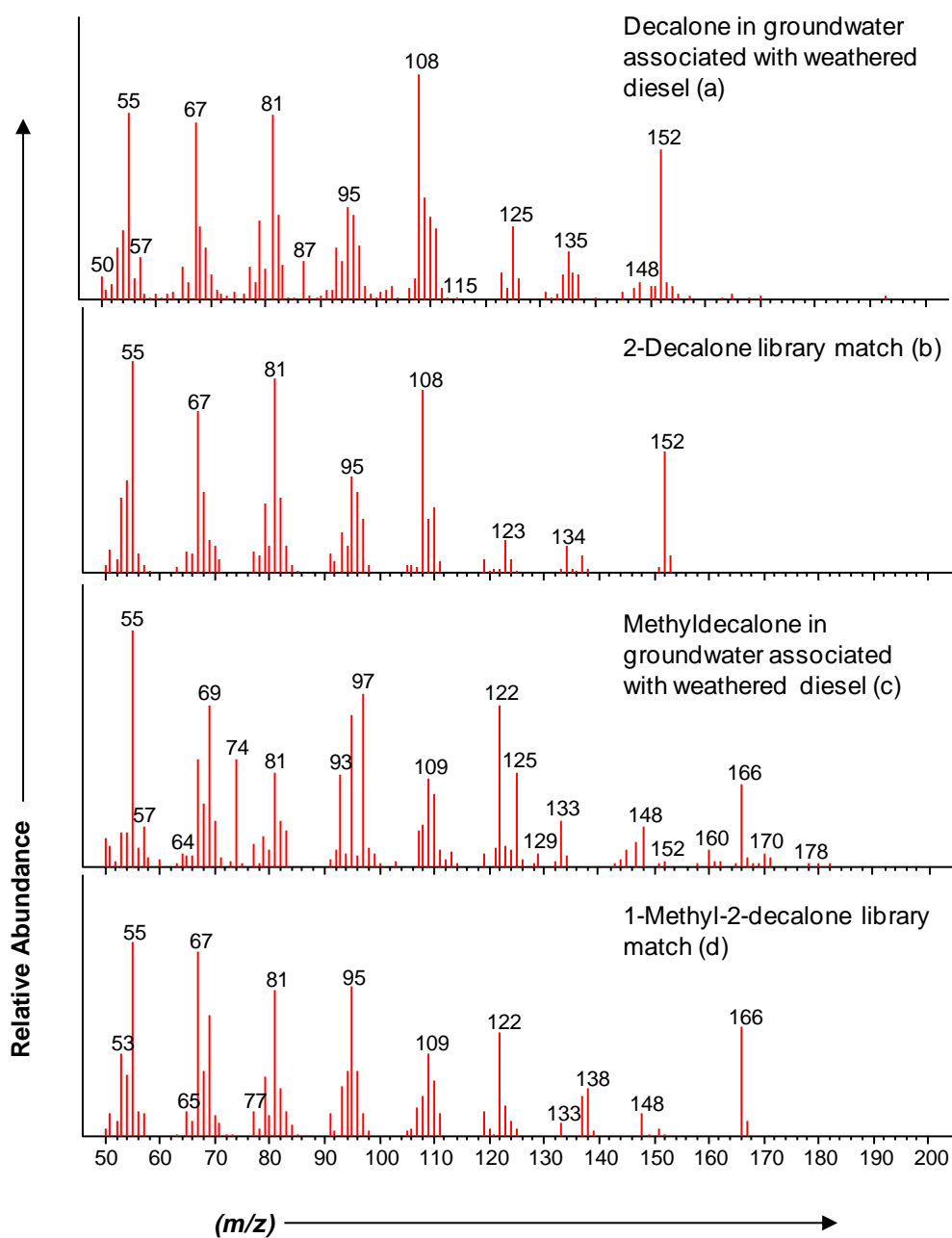


Figure 4.25 Mass spectra for a) a decalone in groundwater associated with weathered diesel, b) library spectrum for 2-decalone, c) a tentatively identified methyldecalone in groundwater associated with weathered diesel and d) library spectrum for 1-methyl-2-decalone .

4.5.2.2.3 Tetralones

Tetralones were identified in soil (Fig. 4.22) and groundwater (Figs. 4.18a, 19a, 26) associated with weathered diesel, as well as water equilibrated with weathered diesel (Figs. 4.18b, 19b). 1-Tetralone and 2-methyl-1-tetralone were identified using authentic standards. A range of methyltetralones were also tentatively identified (Fig. 4.19). The occurrence of these tetralones in groundwater associated with weathered diesel has not been reported, to the knowledge of the author.

Tetralones identified in groundwater associated with weathered diesel are likely formed from the biodegradation of tetralins. Similar to the indanones, the formation of tetralones appears to be limited to aerobic biodegradation processes occurring selectively at the benzylic carbon atom to produce 1-tetralones (Fig. 4.27) (Schreiber and Winkler, 1983; Sikkema and Debont, 1991; Grayson *et al.*, 1996; Limberger *et al.*, 2007; Lie *et al.*, 2009). The anaerobic biodegradation of tetralin has not been shown to form tetralone, alternatively resulting in the formation of a carboxylic acid (5,6,7,8-tetrahydro-2-naphthoic acid) (Annweiler *et al.*, 2002) (Fig. 4.27). This lack of evidence from previous laboratory studies, coupled with the identification of exclusively 1-tetralone, and a limited number of methyltetralin isomers supports the premise that tetralones have formed aerobically.

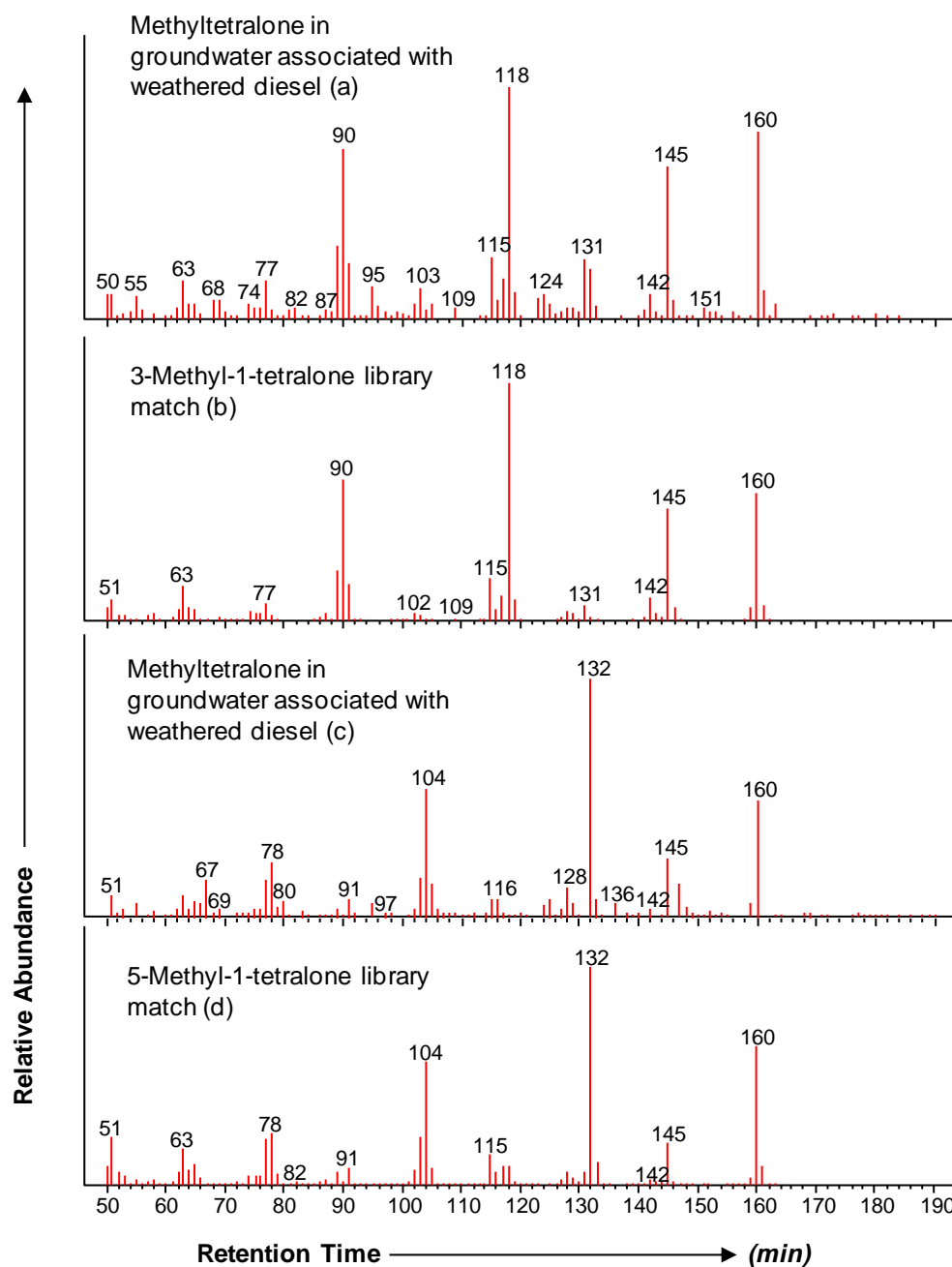


Figure 4.26 Mass spectra for a) tentatively identified methyltetralone (methyl group attached to the cyclic ring) b) library spectrum for 3-methyl-1-tetralone, c) tentatively identified methyltetralone isomer (methyl group attached to the aromatic ring) and d) the library spectrum for 5-methyl-1-tetralone.

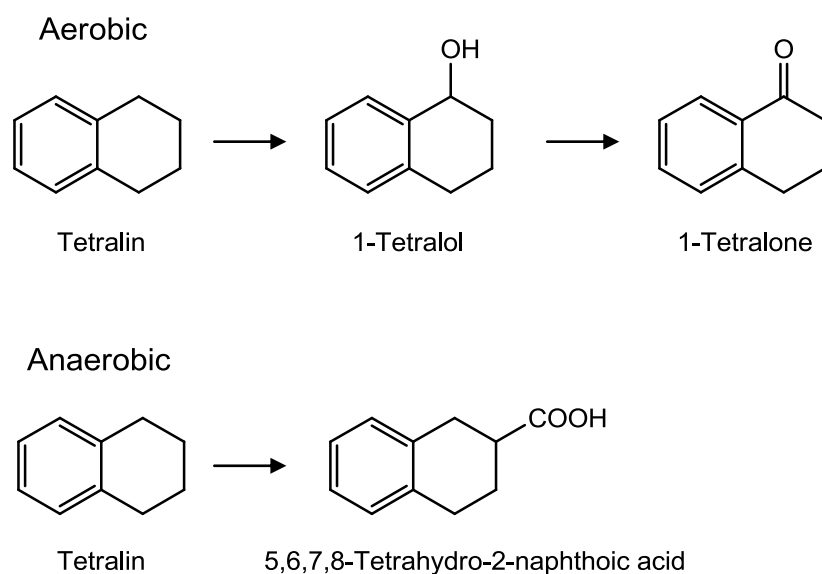


Figure 4.27 Pathways for the aerobic and anaerobic biodegradation of tetralin.

Tetralones were present in the groundwater associated with weathered diesel and water equilibrated with weathered diesel, suggesting they have accumulated in the NAPL and partitioned into the groundwater. Differences in the distributions of tetralones in the groundwater and water equilibration experiments are likely a result of the tetralones undergoing further degradation in the groundwater.

4.5.2.3 Sulfur containing polar compounds

Heterocyclic sulfur compounds containing oxygenated functional groups were tentatively identified in the water equilibrated with weathered diesel (Fig. 4.28a). These included a sulfoxide (2-methyl-1-thiabicyclodecane sulfoxide; Fig. 4.29a) and a thioester (1-thiaacenaphthen-2-one; Fig. 4.29c). The thioester identified may also be a sulfoxide, as this compound would share a similar mass spectrum. This could not be confirmed, as a library match for the sulfoxide configuration of this compound was unavailable.

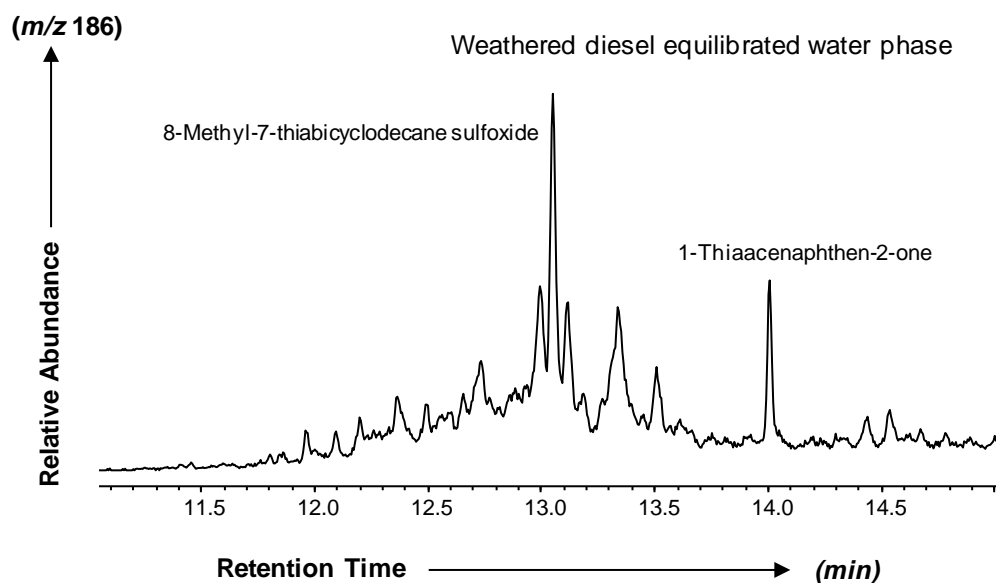


Figure 4.28 Partial ion chromatogram (m/z 186) showing 2-methyl-1-thiabicyclodecane sulfoxide and 1-thiaacenaphthen-2-one in water equilibrated with weathered diesel.

The presence of these heterocyclic sulfur compounds containing oxygenated functional groups is likely a result of the biodegradation of heterocyclic sulfur compounds present in the diesel. Sulfoxides and ketones have been reported to form from the aerobic biodegradation of heterocyclic sulfur compounds such as benzo- and dibenzothiophenes (Kodama *et al.*, 1973; Mormile and Atlas, 1989; Fedorak and Grbicgalic, 1991; Saftic *et al.*, 1992; Kropp *et al.*, 1997a; Seymour *et al.*, 1997). Therefore the presence of 2-methyl-1-thiabicyclodecane sulfoxide and 1-thiaacenaphthen-2-one is likely a result of the aerobic biodegradation of the sulfur containing heterocyclic compounds (2-methyl-1-thiabicyclodecane and 1-thiaacenaphthene, respectively; Fig. 4.30).

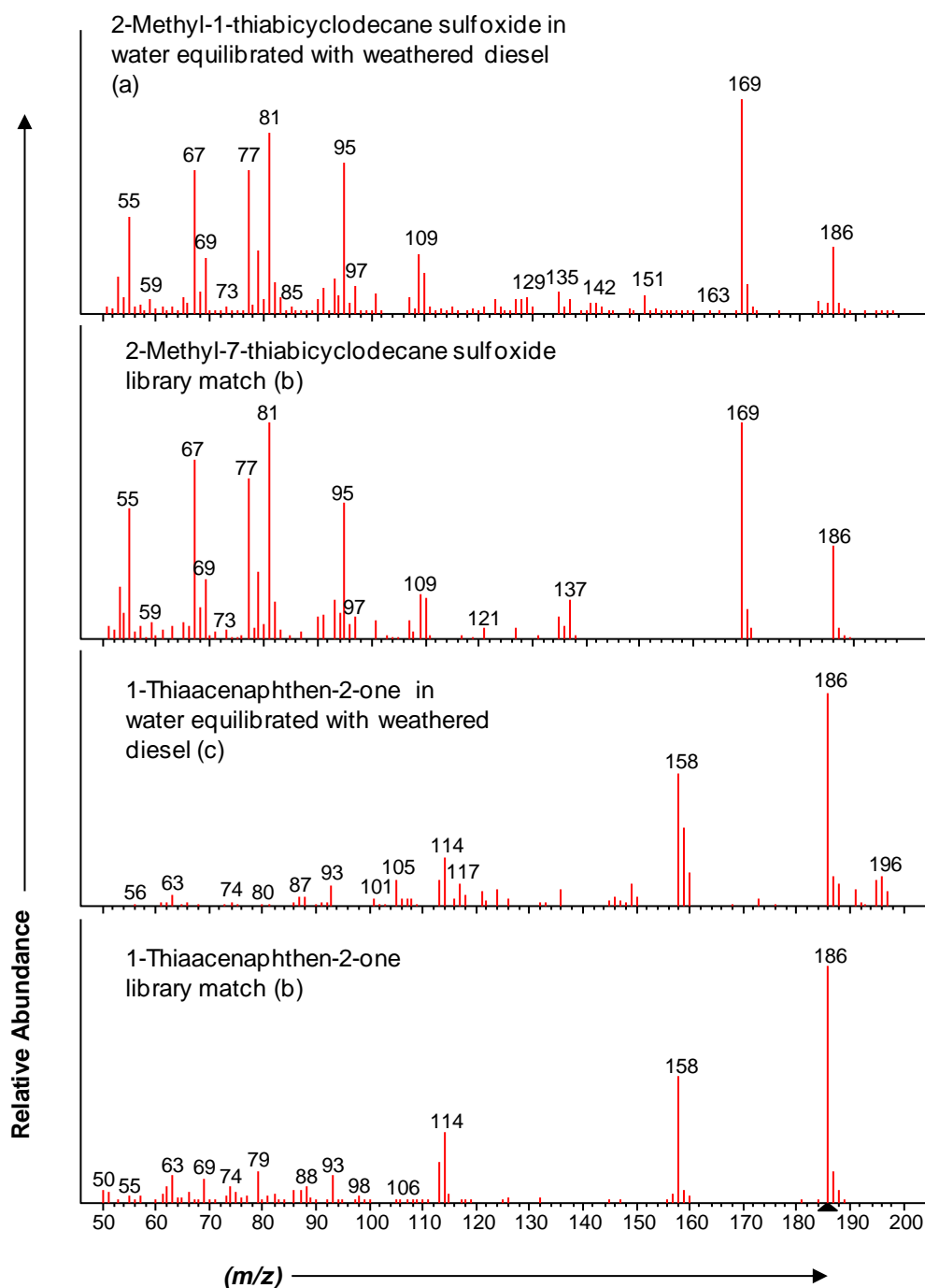


Figure 4.29 Mass spectra for a) tentatively identified methylthiabicyclodecane sulfoxide, b) library spectrum for 2-methyl-7-thiabicyclodecane sulfoxide, c) tentatively identified sulfur containing ketone and d) the library spectrum for 1-thiaacenaphthen-2-one.

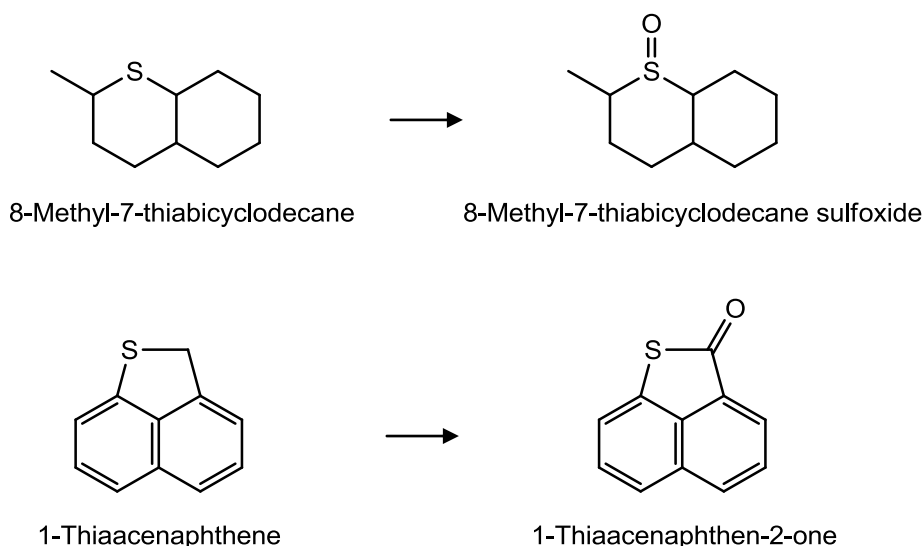


Figure 4.30 **Proposed pathways for the formation of 2-Methyl-1-thiabicyclodecane sulfoxide and 1-thiaacenaphthen-2-one.**

The formation of sulfoxides may not be limited to biodegradation processes, and may alternatively occur through abiotic processes. This has been shown for sulfur containing heterocyclic compounds that are suggested to undergo autooxidation in shallow petroleum reservoirs from exposure to oxic water (Waldo *et al.*, 1991). This has also been suggested to be a possible mechanism for the degradation of sulfur containing heterocyclic compounds present in crude-oil-contaminated groundwater (Thorn and Aiken, 1998). The identification of 2-methyl-1-thiabicyclodecane sulfoxide and 1-thiaacenaphthen-2-one in only the equilibrated water samples suggests these compounds form and accumulate in the weathered petroleum and are rapidly degraded in the groundwater.

4.5.2.4 Carboxylic acids

Polar compounds containing carboxylic acid functional groups encompassed the largest range of compounds in this study. These compounds included benzoic, biphenylacetic, diphenylacetic, naphthoic, naphthaleneacetic, indanoic and tetrahydronaphthoic acids that were only identified in the groundwater associated with weathered diesel.

4.5.2.4.1 Benzoic acids

Benzoic acids identified included C3-alkylbenzoic and C4-alkylbenzoic acids, biphenylacetic acids and diphenylmethanecarboxylic acids, and were only present in the groundwater samples associated with weathered diesel.

C3-Alkylbenzoic acids identified included the 2,4,6-trimethylbenzoic acid (based on an authentic standard) and the tentative identification of two C3-benzoic acid isomers that did not include 2,4,5-trimethylbenzoic acid (an authentic standard for this compound did not match any isomer present) (Figs. 4.31-32).

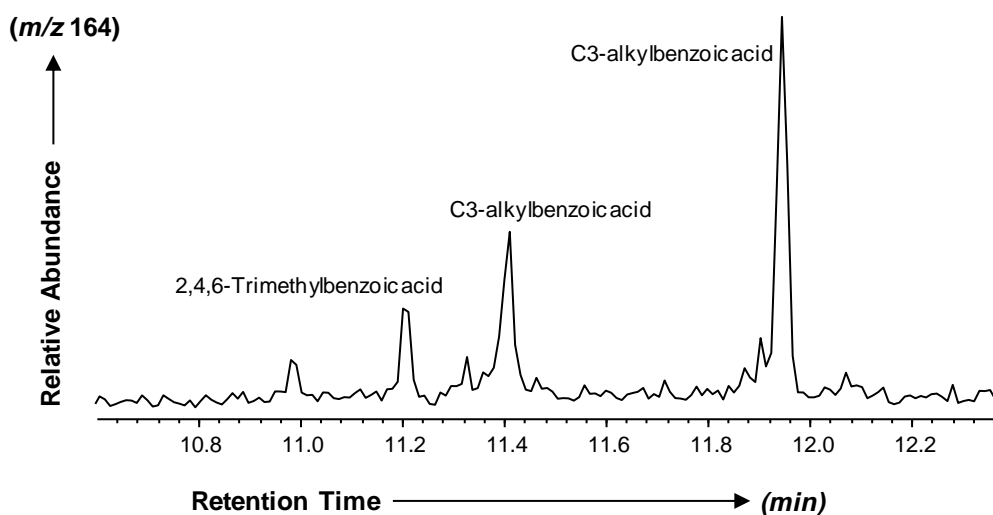


Figure 4.31 Partial ion chromatogram (m/z 164) showing C3-alkylbenzoic acids in a groundwater associated with weathered diesel.

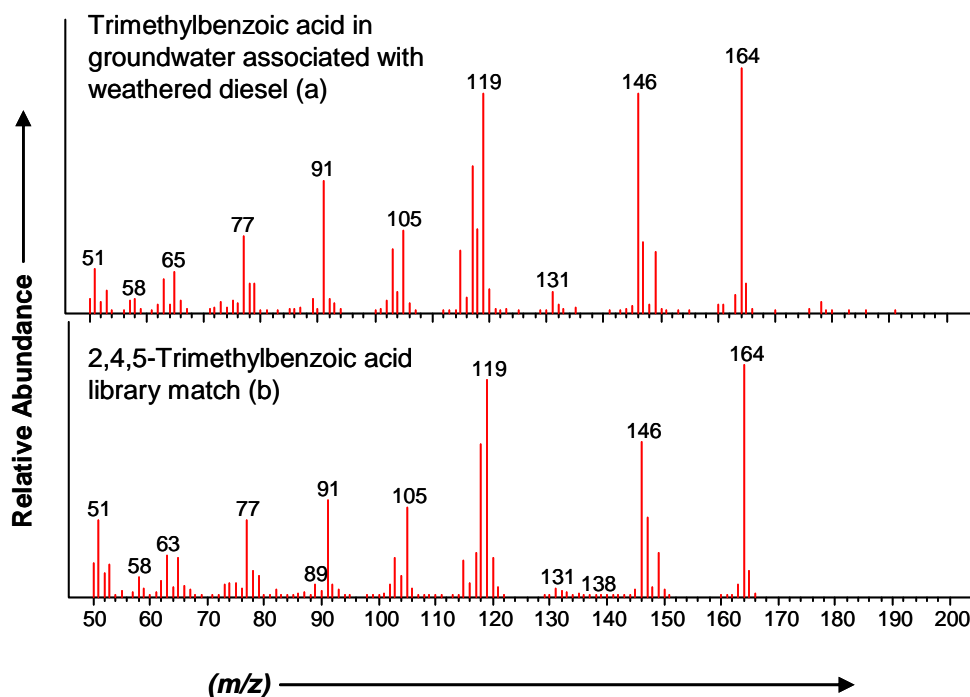


Figure 4.32 Mass spectrum for a) tentatively identified trimethylbenzoic acid and b) the library spectrum for 2,4,5-trimethylbenzoic acid.

Benzoic acids identified in groundwater associated with weathered diesel are likely formed from the biodegradation of alkylbenzenes in the groundwater. Benzoic acids have been previously identified in groundwater associated with diesel (Cozzarelli *et al.*, 1990), gasoline (Cozzarelli *et al.*, 1995), jet fuel (Namocatcat *et al.*, 2003) and unspecified gas condensate (96% w/w C5-C15 compounds; (Gieg *et al.*, 1999) releases. Anaerobic processes have been suggested to be responsible for benzoic acid formation, with trimethylbenzoic acids identified in groundwater suggested to form from the anaerobic biodegradation of tetramethylbenzenes (Fig. 4.33) (Namocatcat *et al.*, 2003). However, the formation of trimethylbenzoic acids from tetramethylbenzenes is also likely to occur aerobically, and through the same pathways described by Namocatcat *et al.* (2003) (i.e. oxidation of the methyl group of an aromatic compound to form an alcohol, followed by further transformation to an aldehyde and carboxylic acid) (Shen *et al.*, 2000; Jindrova *et al.*, 2002).

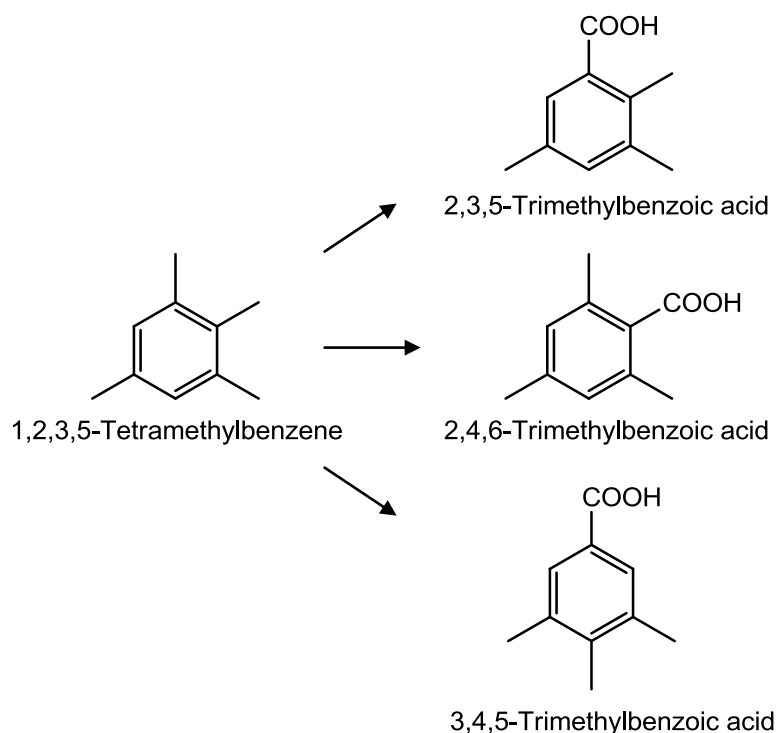


Figure 4.33 Possible biodegradation pathways for 1,2,3,5-tetramethylbenzene. Adapted from Namocatcat et al. (2003).

C3-Alkylbenzoic acids were not detected in water equilibrated with weathered diesel suggesting they are formed in groundwater from the biodegradation of water soluble alkylbenzenes. Biodegradation products that are specific to anaerobic biodegradation (i.e. benzylsuccinic acids; Beller *et al.* 1995) were not identified and it is therefore difficult to determine if the C3-alkylbenzoic acids have formed aerobically at the fringe of the dissolved plume, or anaerobically within the dissolved plume where the groundwater is typically devoid of oxygen due to the rapid depletion of oxygen through aerobic biodegradation (Borden *et al.*, 1995). The groundwater sample contained significant abundances of alkylbenzenes and other aromatic components, suggesting the C3-alkylbenzoic acids have formed from the anaerobic biodegradation of C3-alkylbenzenes in the dissolved plume. The distribution of tetramethylbenzenes in groundwater associated with weathered diesel (Fig. 4.34a) and the recovered weathered diesel (Fig. 4.34b) show that the alkylbenzenes present in the groundwater have been significantly altered due to biodegradation processes, with 1,2,3,5- (the likely parent compound to

2,4,6-trimethylbenzoic acid) and 1,2,4,5-tetramethylbenzene severely depleted. The biodegradation of tetramethylbenzenes in the groundwater samples associated with weathered diesel supports the premise that the alkylbenzoic acids have formed from the biodegradation of the alkylbenzenes in the water phase. However, due to the similar pathways of aerobic and anaerobic biodegradation specified, it is difficult to determine whether the compounds have formed through aerobic or anaerobic processes.

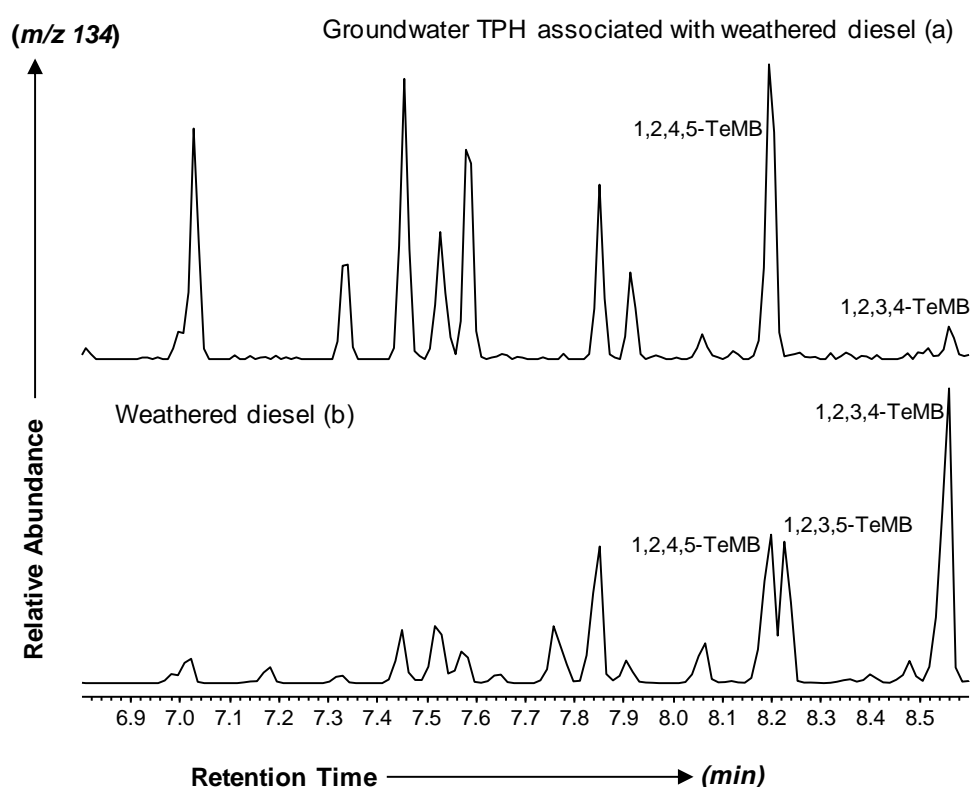


Figure 4.34 Partial ion chromatograms (m/z 134) showing tetramethylbenzenes in a) a groundwater associated with weathered diesel and b) a recovered weathered diesel.

A C₄-alkylbenzoic acid in groundwater associated with weathered diesel (Fig. 4.35) was tentatively identified as a tetramethylbenzoic acid (Fig. 4.36). This compound is likely formed from the biodegradation of a tetramethylbenzene (through carboxylation) or a pentamethylbenzene (oxidation of a methyl group) with the biodegradation taking place in the groundwater. The identification of only one tetramethylbenzoic acid isomer suggests the position of microbial attack has been specific.

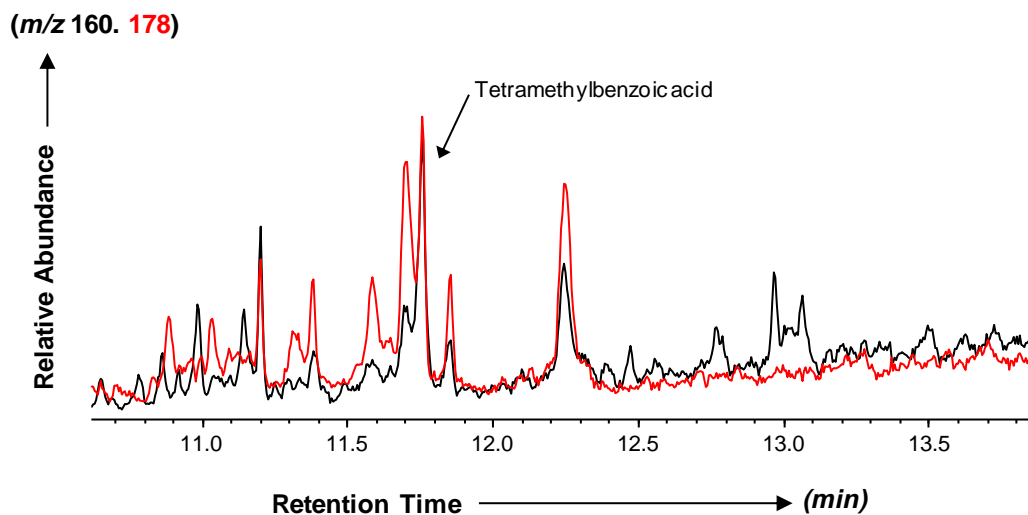


Figure 4.35 Partial ion chromatograms (m/z 160, 178) showing a tetramethylbenzoic acid in a groundwater associated with weathered diesel.

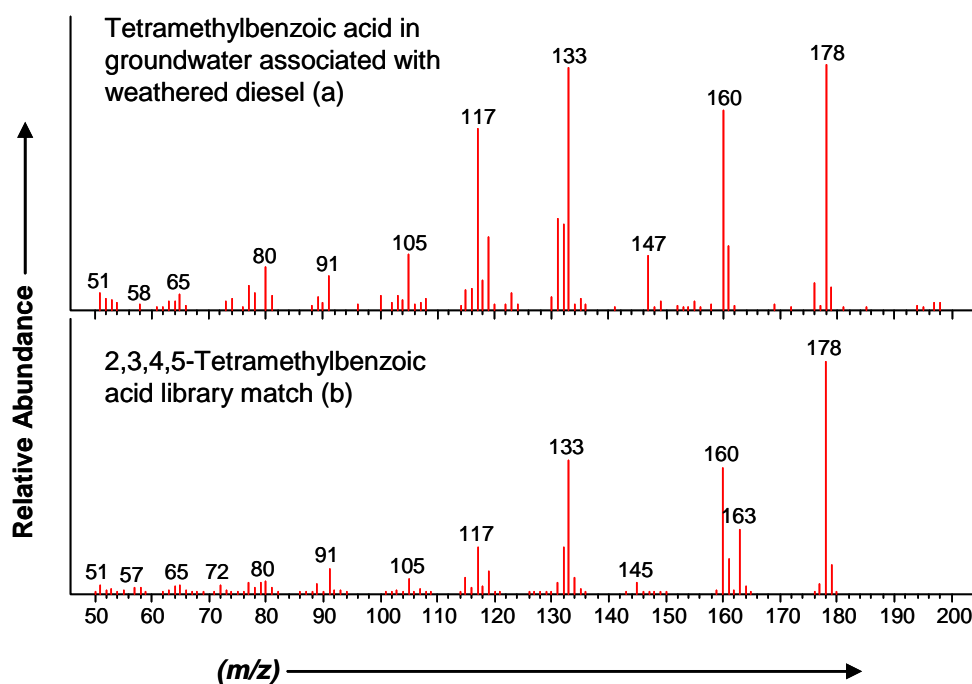


Figure 4.36 Mass spectrum for a) tentatively identified tetramethylbenzoic acid isomer and b) library spectrum for 2,3,4,5-tetramethylbenzoic acid.

4.5.2.4.2 Biphenylacetic, diphenylacetic and phenylmethylbenzoic acids

Carboxylic acid derivatives of biphenyls (biphenylacetic acid) and diphenylmethanes (diphenylacetic and phenylmethylbenzoic acids) were tentatively identified in groundwater TPH associated with weathered diesel (Fig. 4.37). These compounds could not be distinguished due to their similar mass spectral profiles (Fig. 4.38) and the unavailability of authentic reference standards.

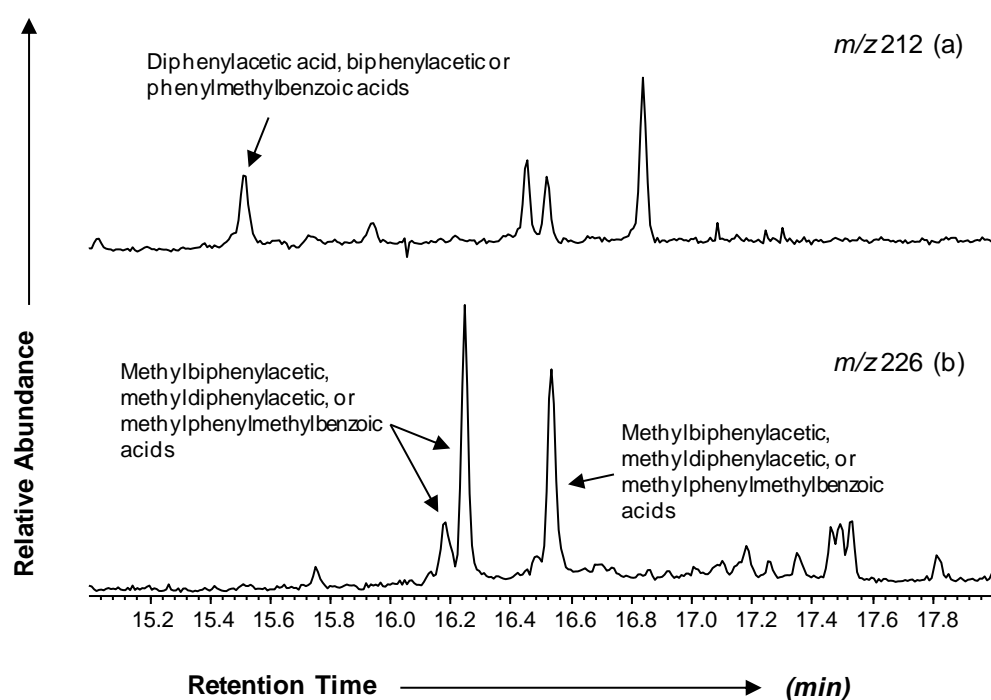


Figure 4.37 Partial ion chromatogram (m/z 212, 226) showing a) diphenylacetic acid, biphenylacetic or phenylmethylbenzoic acids and b) methyldiphenylacetic, methylbiphenylacetic or methylphenylmethylbenzoic acids, in a groundwater associated with weathered diesel.

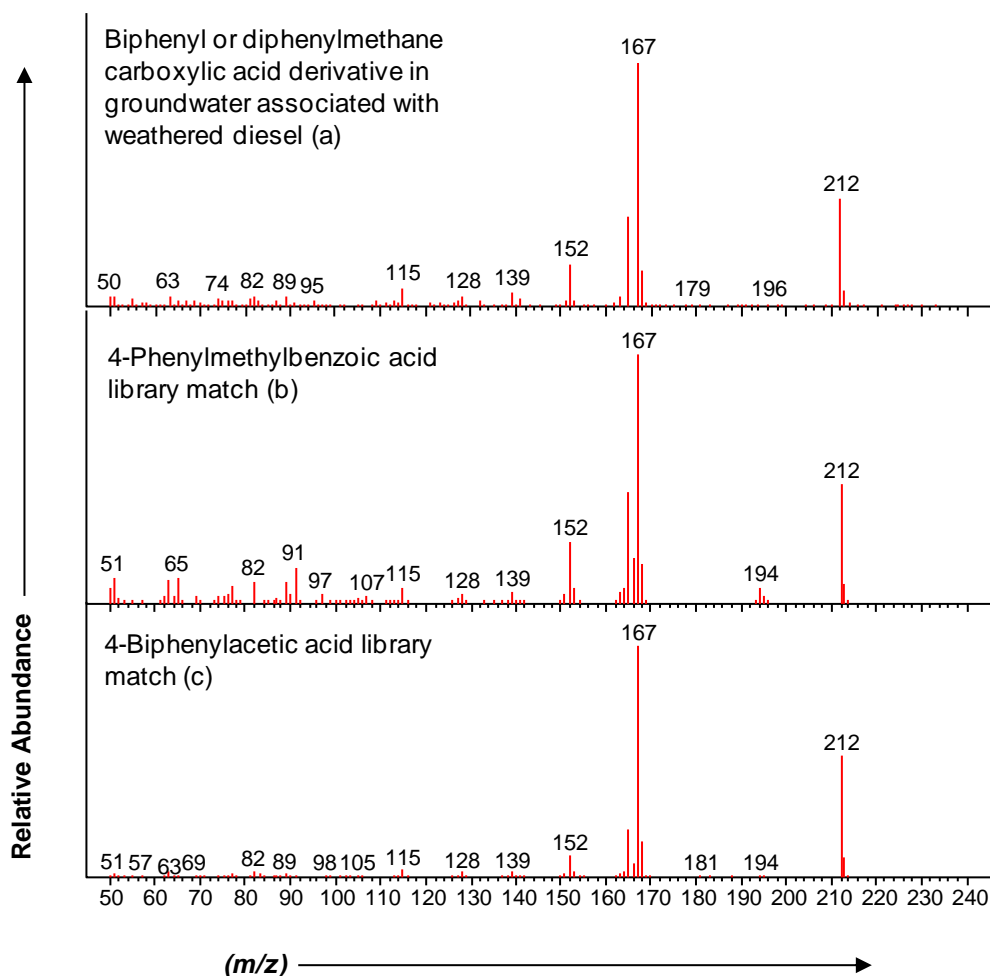


Figure 4.38 Mass spectral profile for a) tentatively identified biphenyl or diphenylmethane carboxylic acid derivative and b) the library spectra for b) 4-phenylmethylbenzoic acid and c) 4-biphenylacetic acid.

The identification of biphenylacetic, diphenylacetic and phenylmethylbenzoic acids in groundwater associated with weathered diesel and not water equilibrated with weathered diesel suggests these compounds are formed from the biodegradation of biphenyls and diphenylmethanes in the groundwater (Fig. 4.39). The likely parent compounds were not identified in the groundwater, suggesting the acids persist after their parent hydrocarbons have been depleted. This resistance to further degradation is possibly a result of these compounds being dead end metabolites at the end of a biodegradation pathway.

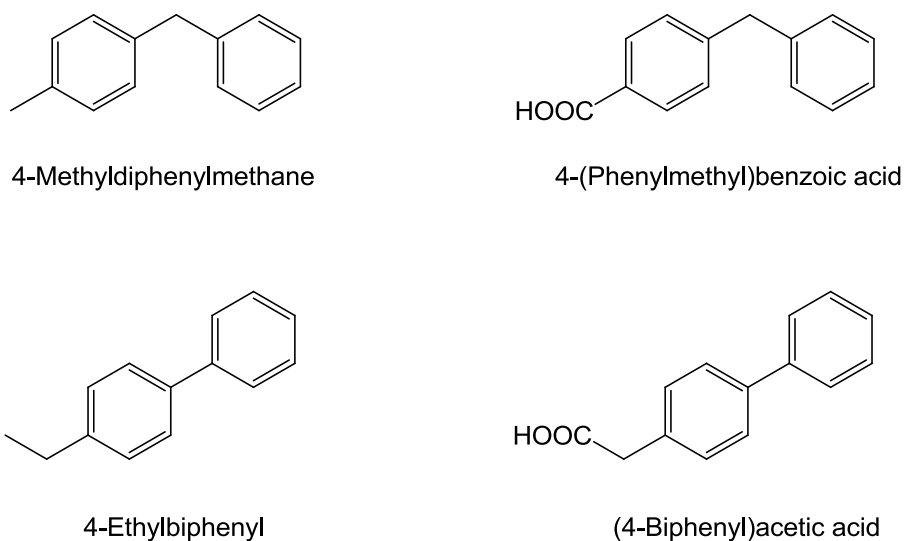


Figure 4.39 Possible biodegradation pathways for 4-methyldiphenylmethane and 4-ethylbiphenyl to form 4-(phenylmethyl)benzoic acid and 4-biphenylacetic acid, respectively.

4.5.2.4.3 Naphthoic and naphthaleneacetic acids

Naphthoic and naphthaleneacetic acids were identified in groundwater associated with weathered diesel (Fig. 4.40). A range of alkylated homologs were tentatively identified based on mass spectra characteristics, though methylnaphthoic and naphthaleneacetic acids could not be distinguished due to their similar mass spectra.

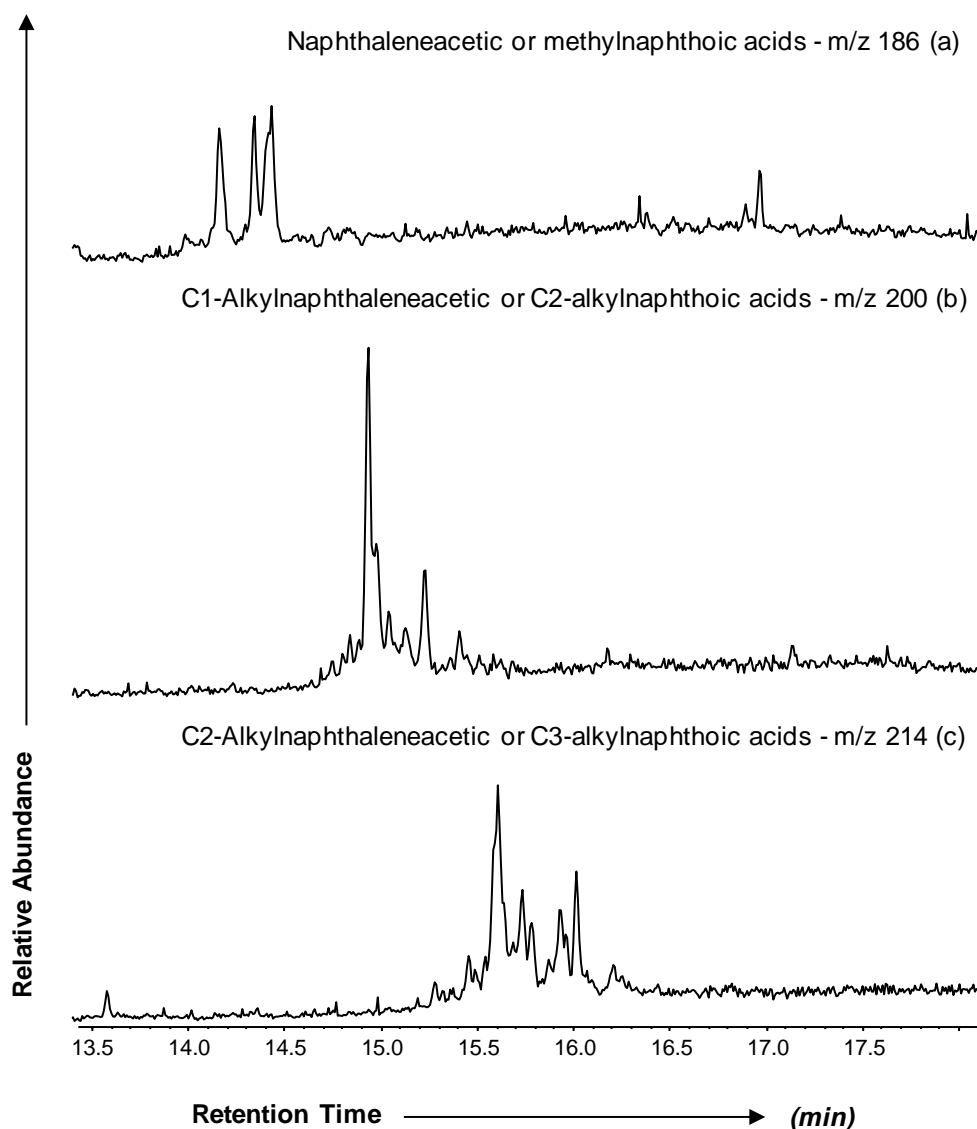


Figure 4.40 Partial ion chromatograms showing a) naphthaleneacetic and/or methylnaphthoic acids (m/z 186), b) C1-alkylnaphthaleneacetic and/or C2-alkylnaphthoic acids (m/z 200), and c) C2-alkylnaphthaleneacetic and/or C3-alkylnaphthoic acids (m/z 214), in groundwater associated with weathered diesel.

Naphthaleneacetic and naphthoic acids are likely formed from the biodegradation of alkylnaphthalenes in groundwater (Fig. 4.41). The presence of these compounds may be due to aerobic and anaerobic biodegradation processes, though their presence in groundwater has been largely associated with anaerobic biodegradation (Annweiler *et al.*, 2002; Meckenstock *et al.*, 2004; Safinowski *et al.*, 2006). No significant changes in

the distributions of dimethyl- and trimethylnaphthalenes due to biodegradation were observed in the groundwater sample.

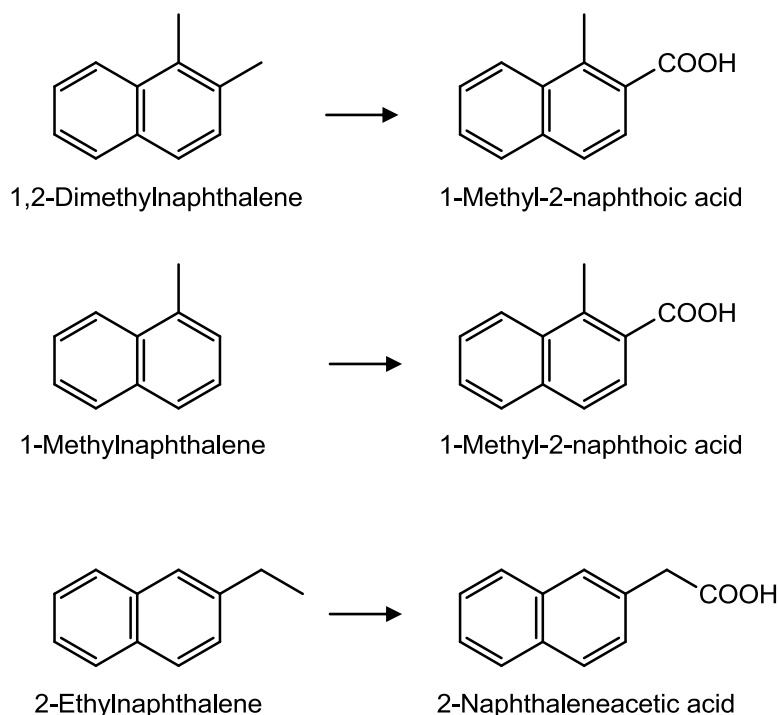


Figure 4.41 Possible biodegradation pathways for 1,2-dimethylnaphthalene and 1-methyl naphthalene to form naphthoic and naphthalene acetic acids.

4.5.2.4.4 Indanoic acids

An indanoic acid was tentatively identified in groundwater associated with weathered diesel (Fig. 4.42) with no alkylated isomers identified. The relatively poor match shown for the indanoic acid in groundwater (Fig. 4.43a) to 2-indanoic acid library spectra (Fig. 4.43b) is likely a result of co-eluting peaks in the underlying polar UCM (i.e. the 144 m/z ion). The presence of this compound is likely a result of its formation from the biodegradation of an indane in the groundwater. Indanoic acids (i.e. 5-indanoic acid) have been previously identified in anoxic groundwater associated with tar oil contamination, which were suggested to form through anaerobic processes (Annweiler *et al.*, 2001).

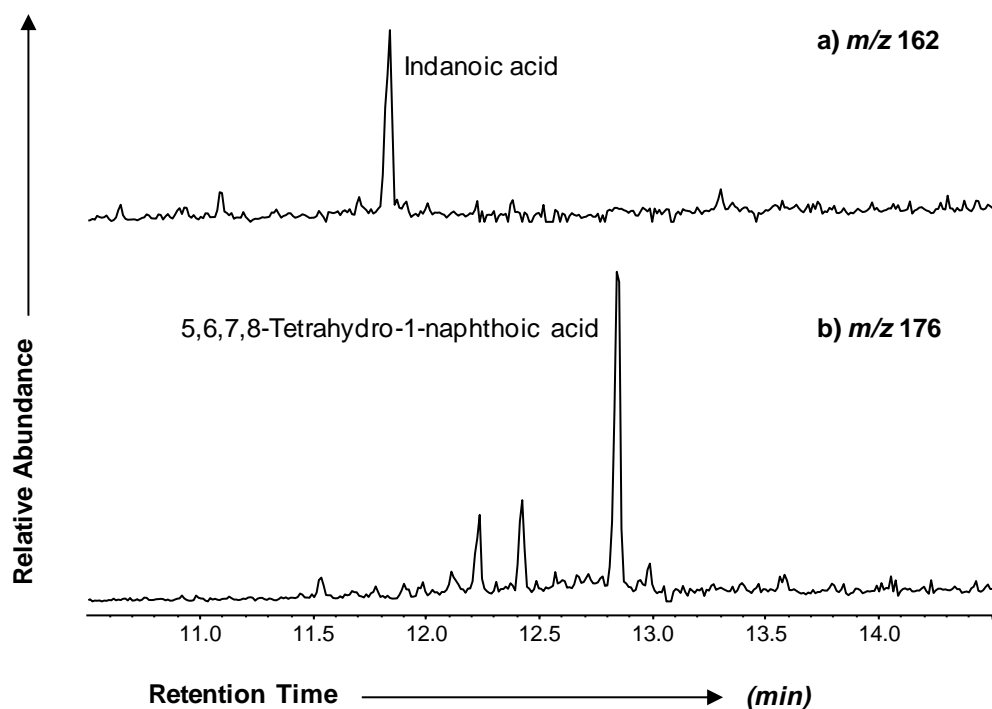


Figure 4.42 Partial ion chromatograms showing a) indanoic (m/z 162) and b) tetrahydronaphthoic acids (m/z 176), in a groundwater associated with weathered diesel.

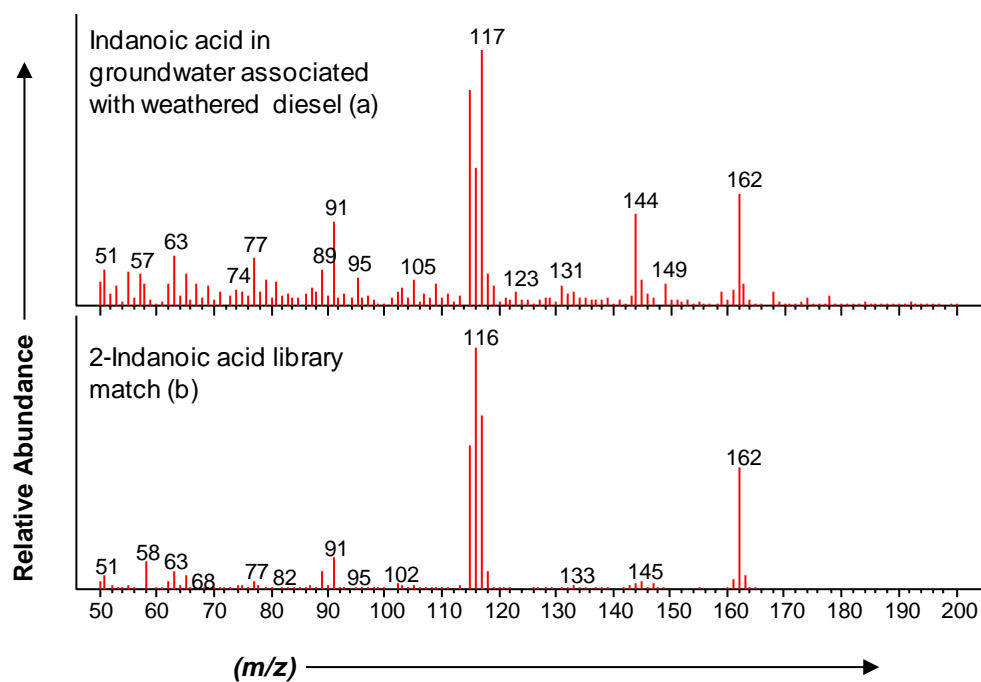


Figure 4.43 Mass spectrum for a) tentatively identified indanoic acid and b) the library spectrum for 2-indanoic acid.

4.5.2.4.5 Tetrahydronaphthoic acids

An unsubstituted tetrahydronaphthoic acid (5,6,7,8-tetrahydro-1-naphthoic acid) was tentatively identified in groundwater associated with weathered diesel, with no alkylated homologs detected (Fig. 4.42b). The tentative identification of 5,6,7,8-tetrahydro-1-naphthoic acid was made on the basis that the other possible isomers (i.e. 5,6,7,8-tetrahydro-2-naphthoic acid and 1,2,3,4-tetrahydro-2-naphthoic acid) were not present in the sample (based on authentic standards). The library mass spectra for 1,2,3,4-tetrahydro-1-naphthoic acid was also significantly different, with 5,6,7,8-tetrahydro-2-naphthoic acid showing a more pronounced 158 ion as a result of dehydration via proton transfer from the *ortho* substituted carbon atom to the hydroxyl group (Fig. 4.44).

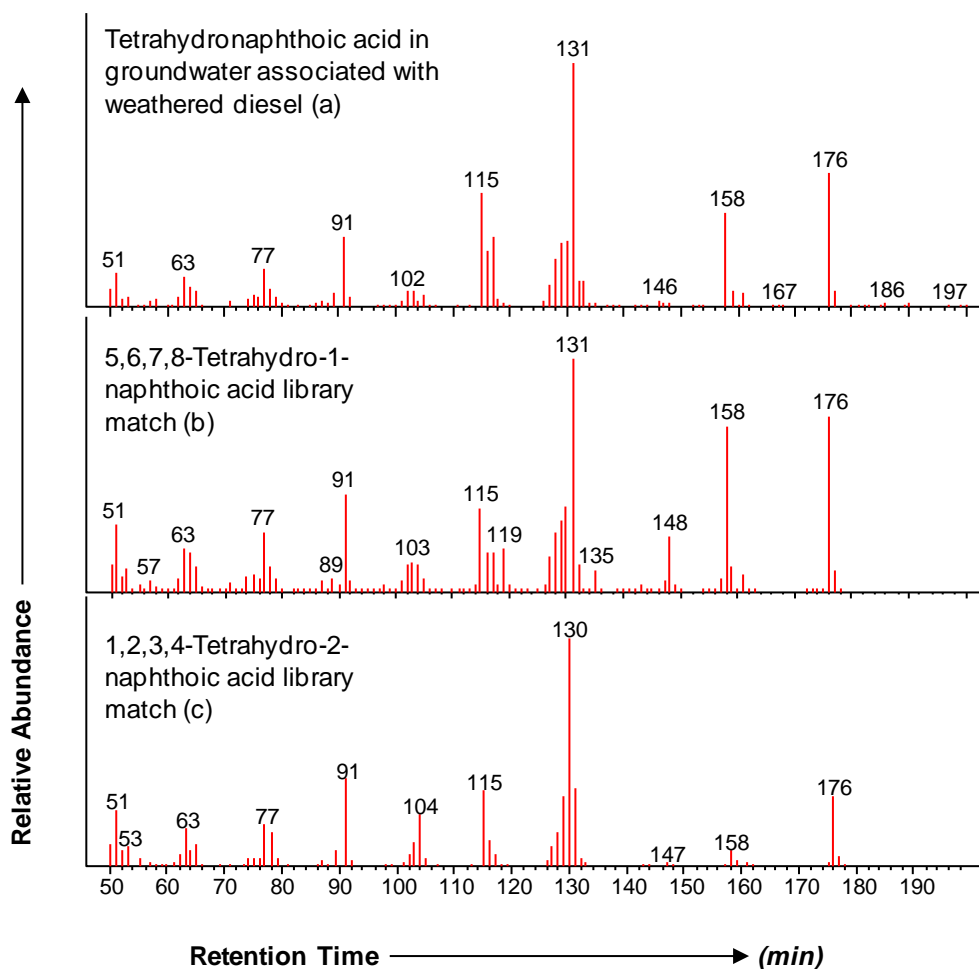


Figure 4.44 Mass spectrum for a) tentatively identified of 5,6,7,8-tetrahydro-1-naphthoic acid, and library spectra for b) 5,6,7,8-tetrahydro-1-naphthoic acid and c) 1,2,3,4-tetrahydro-2-naphthoic acid.

The compound tentatively identified as 5,6,7,8-Tetrahydro-1-naphthoic acid was not identified in water equilibrated with petroleum, suggesting it is formed in the groundwater. This mechanism may involve oxidation of the methyl group of 5-methyltetralin (Fig. 4.45), though no significant changes were observed in the methyltetralin distribution in the groundwater samples compared to the weathered diesel NAPL. Alternatively, the process may be similar to the anaerobic biodegradation of tetralin to form 5,6,7,8-tetrahydro-2-naphthoic acid (Annweiler *et al.*, 2002). However, the formation of the tetrahydro-1-naphthoic acid isomer from tetralin has not been reported, to the knowledge of the author.

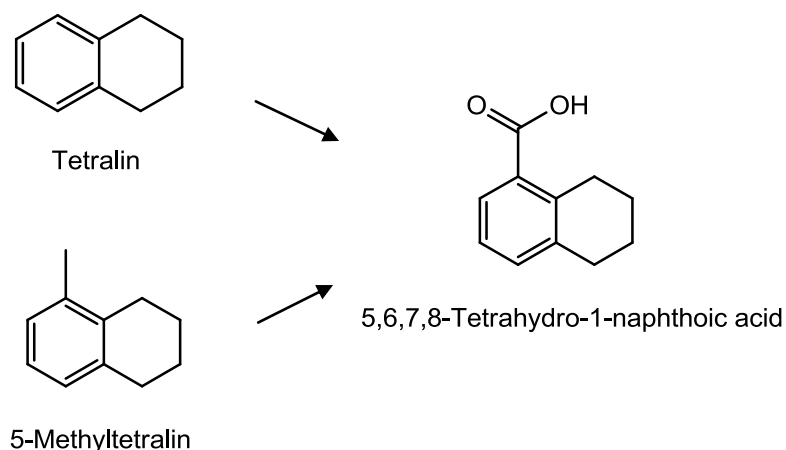


Figure 4.45 Possible transformation pathway for the formation of 5,6,7,8-tetrahydro-1-naphthoic acid from tetralin and 5-methyltetralin.

4.5.3 Polar UCMs

The individual polar compounds identified may provide insights into the composition of polar UCMs associated with weathered petroleum. Polar compound types identified in this study primarily included biodegradation products of petroleum constituents that are resistant to biodegradation (e.g. alkyladamantanes, alkylindanes, alkyltetralins, alkylbenzenes, alkylbiphenyls, diphenylmethanes, and alkylnaphthalenes). Biodegradation products of more readily biodegraded compounds (*n*-alkanes, benzene, toluene, ethylbenzene and xylenes) were not identified, and this likely reflects their higher susceptibility to biodegradation.

The polar compounds identified in the present study all show structural characteristics that demonstrate they have oxidised to their full extent possible without carbon skeleton rearrangement, and this may account for the persistence of these polar compounds and possibly the polar UCMs (as shown in Chapter 2).

4.5.3.1 Mass spectra of polar UCMs

The mass spectra of the polar UCMs associated with weathered petroleum (Fig. 4.46) show the presence of diagnostic ions that may be related to typical carbon skeleton structures. Therefore, assessment of the diagnostic ions present in the polar UCMs may provide some additional information about the compound types present.

Comparison of the averaged mass spectra for polar fractions isolated from soil (Fig. 4.46a), groundwater (Fig. 4.46b) and water equilibrated with weathered diesel (Fig. 4.46c) shows some similarities in the dominant diagnostic ions (i.e. m/z 55, 67, 69, 95, 109, and 123). These ions are common to some cyclic compounds identified in the present study (adamantanols, decalones, and sulfoxides) and potentially a wide range of additional cyclic polar compounds formed from the degradation of petroleum (Fig. 4.47).

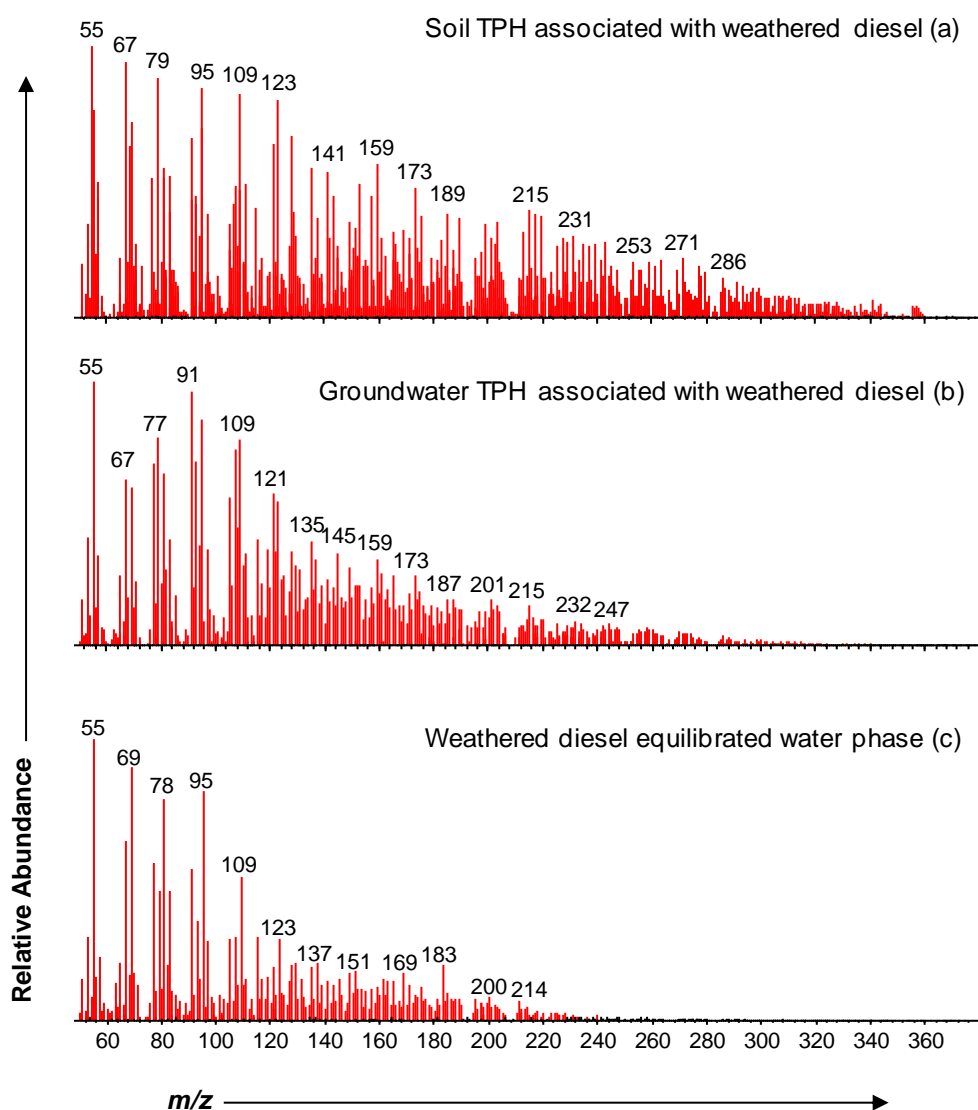


Figure 4.46 Averaged mass spectra for polar UCMs from a) soil and b) groundwater TPH, and c) equilibrated water phase associated with weathered diesel.

The prominence of these ions in the polar UCMs suggests that a significant proportion of these UCMs are composed of cyclic polar compounds. Studies characterising UCMs from biodegraded petroleums have found aliphatic cyclic components to be common constituents (Killops and Aljuboori, 1990), which is likely a result of their increased resistance to biodegradation.

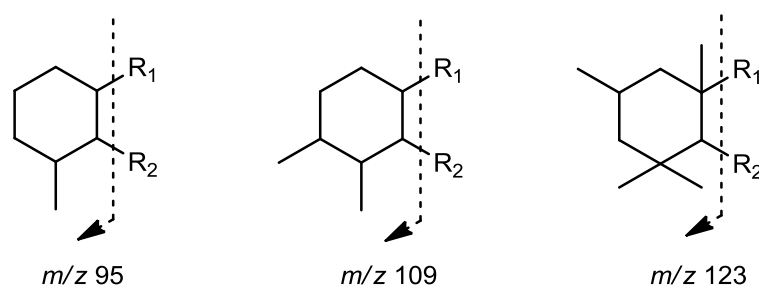


Figure 4.47 Major m/z ions formed from the cleavage of cyclic carbon skeletons

4.6 Conclusions

A range of polar compounds were identified (some for the first time) in soil, groundwater and equilibrated water from weathered petroleum. Major polar compound types identified included tertiary alcohols (i.e. adamantanols), cyclic ketones (i.e. indanones and tetralones) and aromatic carboxylic acids (i.e. benzoic and naphthoic acids). These compounds were identified as biodegradation products of petroleum hydrocarbons, as indicated for some of these compounds by previous field and laboratory studies documenting their formation. Putative parent compounds for these polar compounds are relatively resistant to biodegradation and include compounds with complex cyclic carbon skeletons. The polar compounds identified are also potentially good markers to distinguish between the sources (petroleum and NOM) of polar UCMs, when present.

The presence or absence of individual polar compounds in groundwater associated with weathered diesel and water equilibrated with weathered diesel was used to assess their likely formation process. The occurrence of polar compounds in both groundwater and the equilibrated water phase (alkyladamantanols, indanones, tetralones and sulfoxides, UCMs) suggested they form in the soil profile, accumulate in the NAPL and partition into the associated groundwater. Assessment of the polar component in a soil profile contaminated with weathered diesel showed an increase in polar compounds at the top of a contaminated soil profile suggesting the polar compounds may preferentially form in this aerobic region of the soil profile. In contrast, the

presence of aromatic carboxylic acids in only groundwater suggests they form in the groundwater from the biodegradation of water soluble hydrocarbons, most likely under anaerobic conditions.

The mass spectra of the polar UCMs isolated from soil, groundwater and equilibrated water from weathered diesel were assessed in an attempt to understand the compound types present. The mass spectra of the UCMs showed some similar diagnostic ions to polar compounds containing a range of cyclic carbon skeletons, suggesting that a significant proportion of these UCMs are composed of cyclic polar compounds. The similarities in these mass spectra also suggested that compounds that have accumulated in the weathered diesel are the most significant component of polar UCMs in the groundwater. Therefore, the processes that result in the most commonly reported biodegradation products in groundwater (i.e. aromatic carboxylic acids, forming in the groundwater) may only make a minor contribution to high TPH in groundwater containing predominantly polar compounds as a UCM (e.g. Chapter 2).

5 A novel technique for differentiating petroleum and naturally derived organic matter present as polar unresolved complex mixtures in TPH extracts based on their carbon skeletons

5.1 Abstract

A novel technique was developed to distinguish petroleum and NOM present in total petroleum hydrocarbon (TPH) extracts as polar UCMs. Differentiation was achieved by utilising a dehydrogenation technique to defunctionalise polar compounds and form fewer components (predominantly hydrocarbons) that could be related to petroleum or NOM based on their carbon skeletons. Model polar compounds containing a range of functional groups and carbon skeletons were used to test and optimise the technique (optimum conditions of 280°C, 16hrs, ~6 mg platinum on carbon catalyst per mg model compound). Environmental samples containing polar UCMs from petroleum and NOM (some containing no identifiable hydrocarbons or polar compounds) were used to evaluate the technique, producing reaction products that could be related to their origins. Differentiation of NOM and petroleum polar UCMs was based on the formation of a group of compounds (diamondoids) that have carbon skeleton configurations that only occur in petroleum. Other compounds formed that may be useful to unravelling sources included aromatic compounds formed from the UCMs from NOM with configurations that relate to natural products (e.g. dominant 1,6-dimethylnaphthalene and 1,2,7-trimethylnaphthalene isomers that were related to higher land plant natural products).

The reaction products formed from petroleum also provided insight into the formation, accumulation and composition of the polar UCMs from petroleum. The presence of biodegradation products from compounds that are very resistant (diamondoids) and moderately resistant (alkylbenzenes, alkylnaphthalenes, alkylbiphenyls) to biodegradation suggested that a range of compounds were being biodegraded, rather than the depletion in an apparent step-wise manner based on their relative susceptibilities. This shows that heavily weathered petroleum can undergo further degradation and may represent a significant process in the natural attenuation of severely weathered petroleum. Similarities in the distributions of almost all reaction products formed from the weathered diesel polar fraction and groundwater TPH associated with weathered diesel contamination also suggested the technique may be used to relate polar UCMs to their likely sources. These observations are significant considering some of these polar UCMs offer no characteristic information prior to defunctionalisation.

5.2 Introduction

Differentiating between petroleum and NOM in TPH analyses can be difficult due to the presence of polar UCMs (Girard and Edelman, 1994). NOM incorporated into TPH can result in increased concentrations that do not reflect the actual level of contamination, and in some instances the TPH measured consists of predominantly NOM (Girard and Edelman, 1994). On the other hand, the removal of polar compounds with silica gel based on the premise they are derived from NOM may remove polar compounds that are derived from petroleum and significantly underestimate the actual level of contamination (Lang *et al.*, 2009).

Polar compounds from petroleum that may be present as a UCM in TPH extracts include polar compounds that originate from crude oil (Lundegard and Knott, 2001; Lundegard and Sweeney, 2004) and polar compounds formed from the degradation of petroleum (Zemo and Foote, 2003; Lundegard and Sweeney, 2004). Polar compounds present in crude oils consist of nitrogen, sulfur and

oxygen containing compounds that encompass a wide range of molecular weights (Tissot and Welte, 1984). Polar compounds formed from the biodegradation of petroleum that have been identified in groundwater include alcohol, aldehyde, ketone, and carboxylic acid derivatives of common petroleum analogs (Cozzarelli *et al.*, 1990; Cozzarelli *et al.*, 1994; Beller *et al.*, 1995; Langbehn and Steinhart, 1995; Angehrn *et al.*, 1998; Beller, 2000; Gieg and Suflita, 2002; Ohlenbusch *et al.*, 2002; Namocatcat *et al.*, 2003).

NOM is derived from biochemical precursors that include carbohydrates, proteins, peptides, lipids, amino sugars, lignins and tannins (Thurman, 1985b; Gadel and Bruchet, 1987; Lehtonen *et al.*, 2001; Otto and Simpson, 2007). Polar compounds from NOM that can be incorporated in TPH analyses are potentially diverse due to the varied and complex nature of NOM, ranging from simple organic compounds through to high molecular weight macromolecules (Aiken, 2002). TPH analysis is aimed at the analysis of semi-volatile hydrocarbons and NOM incorporated is therefore likely to be relatively low in molecular weight (i.e. excluding polymeric macromolecules) and polarity (i.e. excluding large polyfunctional molecules). This is a result of the extraction procedure and analytical instrumentation typically used. Compounds from NOM that are likely to be included in TPH include relatively simple polar compounds such as simple organic acids, lipids (e.g. terpenoids) or potentially breakdown products of larger structures (Girard and Edelman, 1994).

Techniques previously used to study NOM and polar compounds related to petroleum include multidimensional GC-MS techniques (Skoczynska *et al.*, 2008; Mao *et al.*, 2009; Melbye *et al.*, 2009) or multi-tiered analytical approaches using a range of instruments (i.e. elemental analysis, FTIR and NMR; Thorn and Aiken, 1998). However, bulk analysis techniques like FTIR and NMR are not likely to provide information that can be used to differentiate compounds that contain a range of functional groups present in a UCM, and multidimensional GC-MS techniques are usually applied to target analytes rather than a UCM. Thermochemolysis techniques such as hydrogenation have been previously used to remove polar functionalities from the polar fraction of crude oil (Sandison *et al.*, 2003). However, this

technique requires a significant amount of material that may not be available from groundwater TPH extracts. Other thermochemolysis techniques such as dehydrogenation have the potential for characterising polar UCMs in TPH extracts, while using only a small amount of sample.

Though differentiating between petroleum and NOM as a UCM can be difficult, fundamental differences exist between their carbon skeletons. For example, carbon skeletons of NOM conform to biosynthetic formation pathways that result in their formation, such as fatty acids and plant waxes that consist of straight chain structures with an even number of carbons atoms due to their initial formation from acetyl units (C₂) (Killops and Killops, 2005). Similarly, terpenoids (a class of lipids that are responsible for a range of complex carbon skeletons found in nature) are comprised of isoprene (C₅) units, leading to specific configurations of cyclic and isoprenoid carbon skeletons (Killops and Killops, 2005).

The range of carbon skeletons present in petroleum is significantly greater than that present in NOM. While petroleum is originally derived from the deposition of NOM, petroleum formation processes result in significant alterations to carbon skeletons and the formation of an extensive range of configurations. This involves high temperature and pressure conditions over millions of years that result in a range of geosynthetic reactions, such as cracking, alkylation, dealkylation, aromatisation and isomerisation reactions that result in the formation of almost every possible carbon skeleton configuration (Tissot and Welte, 1984). For example, *n*-alkanes formed from fatty acids and plant waxes generally show a predominance of an odd number of carbon atoms, whereas the *n*-alkanes in petroleum typically have a smooth distribution with no predominance of odd or even carbon numbers (Volkman *et al.*, 1992; Volkman *et al.*, 1998; Zegouagh *et al.*, 1998). Aromatic compounds present in petroleum also show a range of configurations as a result of the petroleum formation processes, such as the presence of polymethylated naphthalenes with nearly every possible configuration (Alexander *et al.*, 1995; Bastow *et al.*, 1996; van Aarssen *et al.*, 1999; Bastow *et al.*, 2000). The variety of carbon skeletons present in petroleum is

evident by the UCM present in gas chromatograms of petroleum, which contains possibly thousands of individual aliphatic and aromatic hydrocarbons (Blumer *et al.*, 1973; Warton *et al.*, 2000).

Natural product chemists used simple techniques to elucidate carbon skeleton configurations of natural products by transforming them to hydrocarbons (Craig and Jacobs, 1944; Huebner and Jacobs, 1947a; Huebner and Jacobs, 1947b; Sugimoto and Kakimoto, 1959; Kobayashi and Akiyoshi, 1962). These techniques utilised dehydrogenation reactions that defunctionalised and aromatised polar natural product compounds to form aromatic hydrocarbons with much simpler configurations and fewer possible isomers. The identification of the basic ring system of a natural product was an invaluable step in elucidating a product's identity. Dehydrogenation techniques have also been applied to UCMs from petroleum to aromatise saturated cyclic rings, simplifying the UCM by reducing the total number of isomers present (Farcasiu and Rubin, 1987; Gallacher *et al.*, 1991).

The application of a similar dehydrogenation technique to environmental samples containing large polar UCMs may reduce the complexity of the UCMs and produce GC-amenable compounds that can be identified using GC-MS techniques. Dehydrogenation reactions can reduce the complexity of polar compounds by removing their polar functionalities and producing hydrocarbons that are more amenable to analysis by GC. The reduction in complexity is afforded by transforming a range of polar compounds that contain the same carbon skeleton into possibly one hydrocarbon. The identification of the major carbon skeleton types formed may allow identification of the sources for the polar UCMs, which previously offered no characteristic information.

5.3 Scope of study

In this study, an off-line dehydrogenation technique was developed to distinguish petroleum and NOM polar UCMs based on characteristic differences in their carbon skeletons. The technique relies on the

defunctionalisation and aromatisation of polar compounds to produce GC-amenable components (primarily hydrocarbons) that can be related to petroleum and NOM through their carbon skeletons. The technique involves the heating of a sample in the presence of a metal catalyst, resulting in a range of reactions including defunctionalisation, aromatisation, dehydration, reduction (e.g. ketone to alcohol), hydrogenolysis, decarboxylation, dehalogenation, and dehydrocyclisation (e.g. aromatisation and the formation of nitrogen, sulfur and oxygen containing heterocyclic compounds) (Hansch, 1953; Fu and Harvey, 1978). Optimisation of the technique was carried out using model compounds that possess similar carbon skeletons and functional groups to natural products, and petroleum derived polar compounds. The study is also aimed at using the novel technique to characterise polar UCMs associated with petroleum and NOM, to understand the composition of the UCMs that occur in the TPH of environmental samples associated with weathered petroleum.

5.4 Experimental

5.4.1 Model polar compounds

Model polar compounds were obtained from Sigma, Aldrich, Fluka, and TCI, and were AR grade with the exception of abietic acid (75%). These compounds were purchased through the Australian branch of each manufacturer.

5.4.2 Weathered diesel

Weathered diesel used in this study was the same as that used in Chapter 3 (weathered diesel-1). The sample was obtained from a spill release estimated to be up to 50 years old (the same site investigated in Chapter 2 (Johnston *et al.*, 2007) by bailing (~500 mL) the monitoring well.

5.4.3 Groundwater associated with weathered diesel

Groundwater samples (GW-1 and GW-2) were collected from multiport samplers (500 mL) from the same site as the recovered weathered diesel.

5.4.4 NOM water sample

Discoloured water (~4 L) was sampled from a flooded area on the outskirts of Maurie Hamer Park, Herdsman Lake, Perth, Western Australia. As Herdsman lake is a compensation basin for storm water, the occurrence of anthropogenic inputs cannot be discounted. However, water was sampled from a flooded area on the outskirts of the lake (not connected to the lake) where a large source of organic matter was present (Melaleuca trees of the family Myrtaceae, angiosperms).

5.4.5 TPH methods

TPH analysis was carried out using two separate methods. TPH method 1 was used primarily for quantification, and is describe in section 4.4.2.1. TPH method 2 was used to obtain larger amounts of dissolved organics from a 500 mL sample for defunctionalisation without the deuterated surrogate standard which would likely undergo hydrogen exchange during the defunctionalisation technique. Water samples (500 mL) were shaken with DCM (3 x 15 mL) and the recovered DCM dried using anhydrous sodium sulfate. The solvent was reduced to 2 mL by gentle boiling on a sand bath (60°C) affording a sample ready for GC-MS analysis.

5.4.6 Polar compound fractions

Polar fractions were collected using a procedure described by Bastow *et al.* (2007). Degraded diesel (~80 mg) was introduced to a small scale chromatography column and a saturate and aromatic fraction (SAA) eluted under gravity with pentane/DCM (7:3 v/v, 1.8 mL), followed by the elution of the polar fraction (POL) with methanol/DCM (1:1, 1.8 mL). Polar fractions

were gently evaporated to approximately 50 μL , ready for defunctionalisation. Polar compounds from TPH extracts were isolated using the same procedure after the solvent was reduced to 20 μL by gentle evaporation.

5.4.7 Defunctionalisation technique

Defunctionalisation and aromatisation experiments were carried out using selenium, 5 % platinum on Carbon (Pt/C), or 5% palladium on carbon (Pd/C). Model compounds (~0.2 mg dissolved in DCM), model compound mixtures (~1.5 mg of the mixture dissolved in DCM, also containing ~0.2 mg of a surrogate standard *n*-eicosane), a polar fraction from a weathered diesel (~4 mg, dissolved in DCM), and polar fractions from groundwater TPH extracts associated with weathered diesel (GW-1, 7 mg dissolved in DCM; GW-2, 3.5 mg dissolved in DCM) and NOM (2.5 mg dissolved in DCM) were added to glass ampoules (outside diameter 6 mm, wall thickness 1 mm and length ~15 cm) containing the catalyst. The solvent was left to evaporate at room temperature until the catalyst appeared dry. Ampoules were evacuated and flushed with nitrogen three times prior to being flame sealed under vacuum (~3 cm from tube opening). As a safety measure, ampoules were heated in a GC oven (Shimadzu GC-9A series) housed inside a fume hood (when selenium was used as a catalyst) to reduce the risk of the toxic gas hydrogen selenide (H_2Se) being released. A range of reaction times (6 - 24 hrs) and temperatures (220 - 320°C) were used. After cooling to room temperature, ampoules were placed in liquid nitrogen prior to being opened at one end with a glass cutter. The contents were extracted with DCM (2 mL) and filtered through a prewashed plug of cotton wool tightly packed in the stem of a Pasteur pipette affording a sample ready for GC-MS analysis.

5.4.8 Compound class characterisation

Compound class characterisation of defunctionalised products was carried out using the procedure described in Section 2.2.3.

5.4.9 Analysis by Gas Chromatography Mass Spectrometry (GC-MS)

Two GC-MS methods were used in the analysis of samples. Method 1 was used for the TPH analysis and is described in section 4.4.4. Method B enabled detailed separation of the aromatic isomers, and was performed using an Agilent 5975 inert MSD interfaced with an Agilent 6890 gas chromatograph equipped with an Alltech AT-5 ms column (60 m × 0.25 mm i.d., 0.25 µm film thickness). Samples were dissolved in DCM and injected (3 µL) using an autosampler fitted to the vaporising injector. Helium was used as a carrier gas with a linear flow rate of 1 mL/min with the vaporising injector operated in splitless mode. The GC oven was temperature programmed from 35 °C (1 min) to 310 °C (10 min) at 15 °C/min. The MSD was operated with an ionisation energy of 70 eV at 230 °C in scan mode (m/z 50-500).

5.4.9.1 Yields and relative percentages for model compounds and reaction products

Percentages of model compound reaction yields for compound mixtures and single compound reactions were based on TIC responses. Percentages of the original model compound present within the recovered material were calculated for GC-amenable compounds. Yields were based on the actual amount of each model compound (that were GC amenable) in the original mixture, and yields of the product based on the response of the internal standard (d8-naphthalene) using TIC response.

5.4.9.2 Identification of reaction products

Reaction products formed from the defunctionalisation of petroleum and NOM were identified based on published GC retention behaviour and mass spectral characteristics. This includes alkylnaphthalenes (van Aarssen *et al.*, 1999), alkylbiphenyls (Alexander *et al.*, 1986) and alkyladamantanes (Wingert, 1992).

5.5 Results and Discussion

An off-line catalytic dehydrogenation technique was selected to differentiate polar UCMs from NOM and petroleum sources. Development of the technique required the selection of a catalyst, optimisation of reaction conditions using model compounds, and the application of the technique to polar UCMs from NOM and petroleum sources.

5.5.1 Technique development using model compounds

Model compounds were used to develop and optimise the dehydrogenation technique for differentiating polar UCMs from petroleum and NOM. A number of model compounds were selected that contain a range of polar functional groups and carbon skeleton configurations that are likely to occur in petroleum (including degradation products and polar compounds originally present within crude oil) and NOM. Polar compound types included alcohols, aldehydes, ketones, carboxylic acids, methyl esters, ethers and heterocyclic nitrogen and sulfur containing compounds. Carbon skeletons included straight chain, branched chain, cyclic and aromatic structures.

5.5.1.1.1 Optimisation

Optimisation of the technique included the testing of different catalysts, catalyst loadings, reaction temperatures and reaction times using mixtures of model compounds. Optimisation of these parameters was based on limiting carbon skeleton rearrangement/isomerisation whilst maximising desired product (hydrocarbons and heterocyclic compounds) yields.

5.5.1.1.2 Catalyst type

Selenium, Pt/C and Pd/C were tested as dehydrogenation catalysts. Sulfur was discounted as a dehydrogenation catalyst because it can result in sulfur incorporation and the subsequent formation of sulfur containing side products (Fu and Harvey, 1978). These rearrangements are undesirable in the

defunctionalisation technique, as heterocyclic sulfur compound distributions may be characteristic of some petroleum defunctionalisation products. Selenium was tested as a catalyst, as it has been shown that fewer side products are typically observed (Fu and Harvey, 1978).

Model compounds selected for optimising the catalyst type (Table 5.1) included alcohols (2-ethylhexan-1-ol, 1-adamantanol), ketones (2-methylcyclohexanone, and benzophenone), and a carboxylic acid (2,4,6-trimethylbenzoic acid). Experiments were carried out using a relatively moderate temperature (270°C) compared to previously documented dehydrogenations with these catalysts (Fu and Harvey, 1978; Bastow *et al.*, 2001). A lower temperature was likely to reduce isomerisation/alkylation processes. A reaction time (16 hours) that allowed experiments to be carried out over night was used. This reaction time was longer than those typically used for dehydrogenation, and should allow for reactions to reach completion. To assess the effectiveness of each catalyst, the percentage of the model compound, products (hydrocarbons formed with no structural rearrangements), partial products (compounds not completely defunctionalised), and side products (compounds with some structural rearrangement) within the recovered material from each experiment were identified. The relative percentages of the recovered material are shown in Table 5.1.

Table 5.1 Relative percentages for the model compounds, products, partial products and side products recovered from the defunctionalisation of selected model compounds with Se, Pt/C and Pd/C catalysts at 270°C over 16 hours.

Catalyst	Model Compound (%)	Products (%)	Partial products (%)	Side products (%)
Selenium	96	4	0	0
Pd/C	53	32	1	15
Pt/C	50	39	4	8

Selenium was found to be the least suitable catalyst under these conditions, with the lowest percentage of defunctionalised products (4 %). Pt/C had the highest percentage of products (39 %) with the lowest percentage of model compound remaining (50 %) and was therefore considered to be the most appropriate catalyst for the defunctionalisation technique.

5.5.1.1.3 Reaction temperature

A range of temperatures (220°C to 320°C) were investigated using Pt/C as a catalyst with a reaction time of 16 h to determine the optimum temperature (i.e. the temperature where substantial defunctionalisation occurs without significant structural rearrangements). The model compounds used for temperature optimisation included a range of functionalities and carbon skeletons and were combined in a mixture to reduce the number of experiments required. Model compounds were GC-amenable (allowing the relative percentage of the model compounds remaining to be determined) and included alcohols (2-ethylhexan-1-ol, 1-adamantanol, 1-undecanol and 4-isopropylphenol), aldehydes (tetradecylaldehyde), ketones (2-methylcyclohexanone, 2-dodecanone, benzophenone and 9-fluoranone), carboxylic acids (2,4,6-trimethylbenzoic acid and *trans*-4-pentylcyclohexanoic acid), ethers (2,6-dimethoxytoluene) and heterocyclic compounds (dibenzothiophene and carbazole). Relative percentages of model compounds, products, partial products and side products formed are listed in Table 5.2.

Table 5.2 **Relative percentages for the model compounds, products, partial products and side products recovered from the defunctionalisation/aromatisation of model compounds at different temperatures over 16 hours using a Pt/C catalyst.**

Temp (°C)	Model compound (%)	Products (%)	Partial products (%)	Side products (%)
220	65	19	6	10
240	49	36	7	8
260	27	62	6	5
280	24	70	3	3
300	13	84	0	3
320	10	85	1	4

A higher proportion of products and a lower proportion of starting material, partial products and side products were observed at higher temperatures. Though not presented in Table 5.2, temperatures higher than 280°C produced more alkylation products as well as an increased amount of heteroatom removal from carbazole and dibenzothiophene. Lower temperatures produced other higher molecular weight side products that were more favourable than the alkylation products. Defunctionalisation at 280°C formed an adequate percentage of products (70 %), while limiting alkylation and heteroatom removal. As a result, 280°C was selected as the optimum temperature for the defunctionalisation technique.

5.5.1.1.4 Catalyst loading

Pt/C catalyst loadings were optimised (using the same model compounds used for the temperature optimisation) to ensure the quantity of catalyst was not the limiting factor in the defunctionalisation reactions. Catalyst loadings (0.8 – 10.5 mg Pt/C per mg model compound) were used at the optimum temperature (280°C) for 16 hours. The total recovery was also calculated to determine any loss due to an increased amount of catalyst. The total

recovery and relative percentages of model compounds, products, partial products and side products recovered are shown in Table 5.3.

Table 5.3 Percentage recovery, relative percentage of model compounds remaining, products, partial products and side products from the defunctionalisation of model compounds at 280°C with different catalyst loadings for 16 hours using a Pt/C catalyst.

Loading (mg Pt/C per mg model compound)	Model compound (%)	Products (%)	Partial products (%)	Side products (%)	Recovery (%)
0.8	57	19	7	17	71
1.5	52	30	8	10	60
2.9	48	37	11	4	49
5.9	54	33	10	3	47
10.5	44	46	4	6	20

The minimum quantity of catalyst tested (0.8 mg Pt/C per mg model compound) had the lowest percentage of products (19 %), whereas the maximum amount of catalyst tested (10.5 mg Pt/C per mg model compound) produced the highest percentage of products (46 %). Side products (mainly polymerisation products of components in the compound mixture) generally decreased as the catalyst amount increased, with the lowest percentage of side products formed with 5.9 mg Pt/C per mg model compound. Although the maximum amount of catalyst tested formed the greatest percentage of products, the percentage recovery of organic material was significantly lower (20 %) than the experiments with lower catalyst loadings (recoveries of 49 and 47 % for catalyst loadings of 2.9 and 5.9 mg Pt/C per mg model compound respectively). Based on this, Pt/C catalyst loadings of 3-6 mg Pt/C per mg model compound were considered optimum for defunctionalisation on the basis they demonstrated an adequate percentage of products (33 - 37 %), a low percentage of side products (3 - 4 %) and moderate recoveries (47 - 49 %).

5.5.1.1.5 Reaction time

Reaction times ranging from 6 to 24 hrs were tested to determine the optimum time for defunctionalisation. This was carried out using the model compound mixture used for temperature and catalyst loading optimisation, and the previously determined optimum temperature (280 °C) and catalyst loading (6 mg Pt/C per mg model compound). Relative percentages for the model compounds, products, partial products and side products formed are listed in Table 5.5.

Table 5.4 **Relative percentages of the model compounds, products, partial products and side products recovered from the defunctionalisation/aromatisation of model compounds at 280°C over different time periods using a Pt/C catalyst.**

Time (hrs)	Model compound (%)	Products (%)	Partial products (%)	Side products (%)
6	30	62	6	2
12	37	52	9	2
24	29	62	7	2

An extended reaction time was shown to have little effect on the proportion of reaction products formed, with similar percentages shown over 6 and 24 hour time periods. As a result, a reaction time greater than 6 hours (16 hours) was used for all further defunctionalisation experiments which ensured reaction completion, and the convenience of the reactions being performed overnight.

5.5.1.1.6 Individual model compound reaction products

Reactions using individual compounds were carried out to qualitatively identify reaction products without interferences. Model compounds were defunctionalised individually using optimised conditions (~6 mg Pt/C per mg model compound, 280 °C, 16 hours) to identify the major products formed. Relative percentages of the model compound remaining and products formed were based on the TIC response of each compound and are listed in Table 5.5. Some compound types underwent similar reactions and are therefore discussed together.

Cyclic alcohols, ketones and carboxylic acids

Cyclic alcohols (2-ethylcyclohexan-1-ol, 1-adamantanol) carboxylic acids (4-pentylcyclohexanecarboxylic acid, abietic acid) and ketones (2-methylcyclohexanone) all underwent defunctionalisation and aromatisation, except 1-adamantanol (as the carbon skeleton of adamantane cannot aromatise due to its caged structure) (Table 5.5). Other reactions observed for these compounds included a shift of an angular methyl group during the defunctionalisation/aromatisation of abietic acid, and the dehydrocyclisation of 2-ethylcyclohexanol.

2-Ethylcyclohexanol formed the hydrocarbon ethylbenzene (44 %) as the major reaction product. The polar compound benzofuran (23 %) was formed from aromatisation of the cyclic ring and dehydrocyclisation to form an additional heterocyclic ring. 2-Ethylphenol (16 %) was also formed from aromatisation as a minor product.

1-Adamantanol formed adamantane (30 %) as the only hydrocarbon from defunctionalisation. 1-Chloroadamantane (66 %) was an unexpected product formed, which could have resulted from residual chloride from the catalyst (the Pt/C catalyst is produced from chloroplatinic acid) or a result of exchange of chloride from DCM.

2-Methylcyclohexanone formed *o*-cresol (88 %) as the major reaction product, demonstrating aromatisation without complete defunctionalisation (with the ketone group reduced to an alcohol). The defunctionalised and aromatised product toluene (4 %) was formed only as a minor product, suggesting cyclic ketone functionalities are difficult to completely defunctionalise under the conditions.

trans-4-Pentylcyclohexanoic acid aromatised and defunctionalised to form pentylbenzene (18 %) as the major reaction product. 1-Methylnaphthalene (2 %) was formed as a minor product from the cyclisation of the alkyl group. Cleavages to alkyl groups were also shown with the formation of butylbenzene (<1 %), ethylbenzene (<1 %) and toluene (<1 %).

Abietic acid defunctionalised and aromatised to form retene (51 %) as the major product. Methylated retene isomers were also observed, that were likely formed as a result the angular methyl group shifts to adjacent carbon atoms upon defunctionalisation/aromatisation of the cyclic structure (Fu and Harvey, 1978, Bastow *et al.*, 2001b). Cleavage products of abietic acid included 1,2,3-trimethylbenzene (6 %), 1,7-dimethylphenanthrene (5 %) and 1,2,5-trimethylnaphthalene (1 %), which were formed with no significant alkylation/isomerisation products.

Phenols and aromatic ketones, esters, and carboxylic acids

An aromatic ether (2,6-dimethoxytoluene) and a carboxylic acid (2,4,6-trimethylbenzoic acid) defunctionalised to form aromatic hydrocarbons (Table 5.5). Changes to the carbon skeletons of aromatic compounds were only minor and included the cleavage of methyl groups to form minor products. Phenols and ketones on the other hand did not undergo significant defunctionalisation and aromatisation.

2,4,6-Trimethoxybenzoic acid underwent decarboxylation to form 1,3,5-trimethylbenzene (93 %). 2,6-Dimethoxytoluene defunctionalised through the removal of the methoxyl groups to form toluene (33 %) as a major product.

4-Isopropylphenol produced only a minor amount of its defunctionalised hydrocarbon isopropylbenzene (<1 %), remaining largely unchanged (74 % of the model compound recovered). Similar results were shown with 9-fluoranthrone, producing only a low amount of its defunctionalised hydrocarbon biphenyl (1 %), and remaining largely unchanged (9-fluoranthrone consisted of 99 % of the recovered material).

Straight chain primary alcohols, aldehydes, carboxylic acids and esters

Straight chain aldehydes (tetradecanal) and carboxylic acids (tetradecanoic acid and oleic acid) underwent cyclisation and aromatisation during defunctionalisation to form a range of aromatic hydrocarbons (Table 5.5). These products included alkylbenzenes and alkylbiphenyls. The straight chain aldehyde also defunctionalised to form an *n*-alkane. Defunctionalisation of straight chain primary substituted polar compounds largely resulted in the formation of hydrocarbons with one carbon less than the parent compound, as a result of decarbonylation or decarboxylation.

Tetradecanal formed the aliphatic hydrocarbon tridecane (32 %) as the major product. Cyclisation and aromatisation reactions resulted in the formation of C7-alkylbenzenes (48 %). Disubstituted alkylbenzene isomers were formed specifically with the *ortho*-configuration, which is believed to result via the proposed cyclisation mechanisms (Lang *et al.*, 2005). Further cyclisation and aromatisation resulted in the formation of 2-methylbiphenyl (5 %), which is also believed to follow the proposed cyclisation mechanisms (Bastow *et al.*, 2001a).

Tetradecanoic acid formed aromatic hydrocarbons as the major products, though much of the compound remained unchanged (96 %). Products formed included C7-alkylbenzenes with *ortho*-configurations (2 %) as well as 2-methylbiphenyl (2 %). Oleic acid formed similar aromatic hydrocarbons upon defunctionalisation, forming C9-alkylbenzenes (19 %) and C5-alkylbiphenyls (12 %) with specific configurations (i.e. *ortho*- configuration for disubstituted alkylbenzenes and the 2,2'-configuration for alkylbiphenyls). Further cleavage of these alkylbenzene and/or alkylbiphenyls formed form a

significant proportion of benzene (46 %). Methyl hexadecanoate formed hexadecanoic acid as the major product (95 %) as well as C9-alkylbenzenes (2 %) and C2-alkylbiphenyls (2 %) from cyclisation and aromatisation.

Straight chain secondary and branched chain primary alcohols

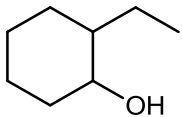
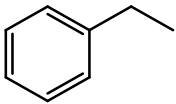
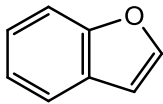
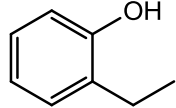
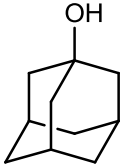
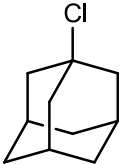
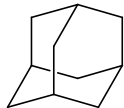
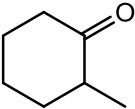
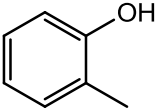
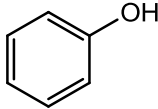
The secondary alcohol (2-dodecanol) did not show significant defunctionalisation, forming 2-dodecanone (96 %) as the major product (Table 5.5).

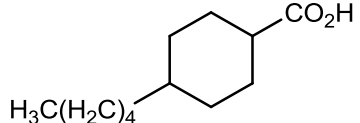
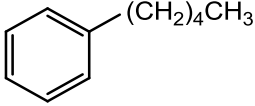
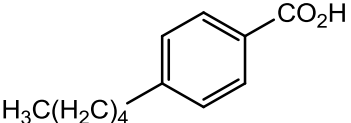
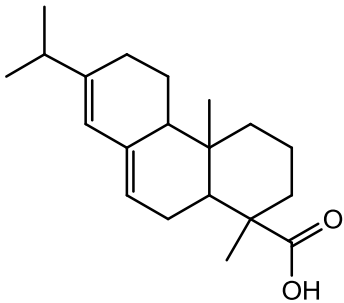
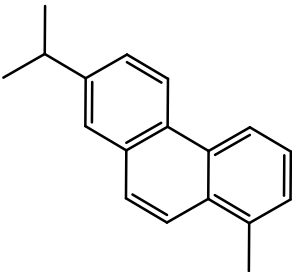
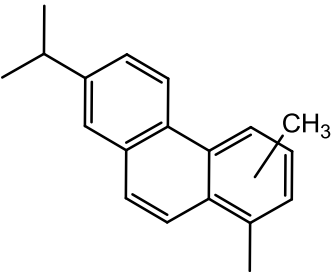
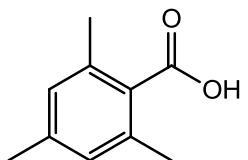
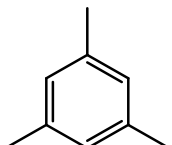
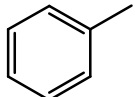
The branched chain (isoprenoid) primary alcohol phytol underwent defunctionalisation and aromatisation to form aliphatic and aromatic hydrocarbons. This included pristane (10 %) and lower molecular weight branched compounds (11 %) formed through defunctionalisation and cleavage of the aliphatic structure. Cyclisation and aromatisation products included *m*- and/or *p*-xylene (34 %) as the major product, as well as alkylbenzene isomers (22 %) formed with only a limited number of isomers, which suggested no significant rearrangements (other than cyclisation and aromatisation) had occurred.

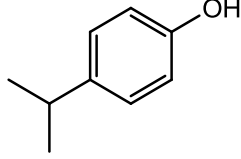
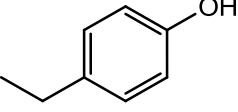
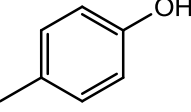
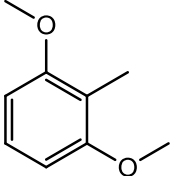
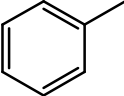
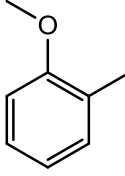
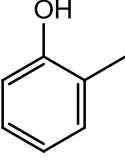
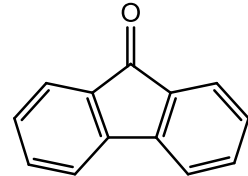
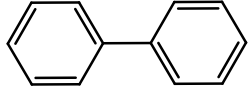
Heterocyclic sulfur and nitrogen containing compounds

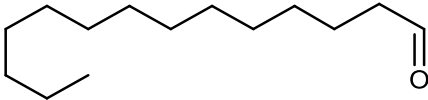
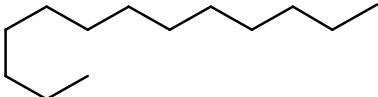
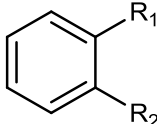
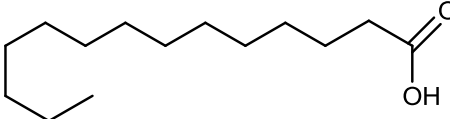
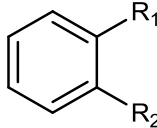
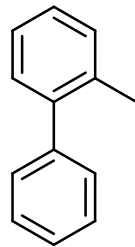
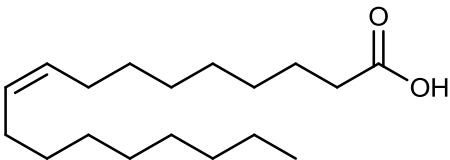
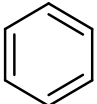
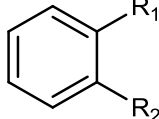
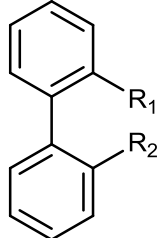
Defunctionalisation of heterocyclic compounds (dibenzothiophene and carbazole) showed that only dibenzothiophene was susceptible to the removal of the heteroatom under these conditions, forming biphenyl (11 %) (Table 5.5)

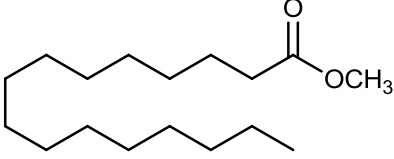
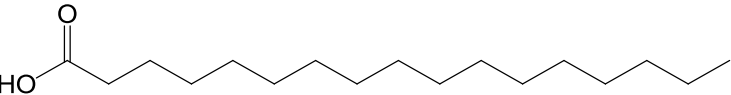
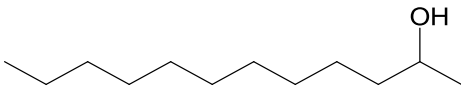
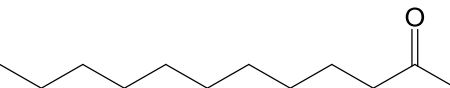
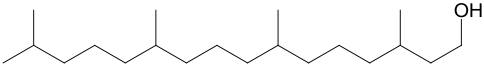
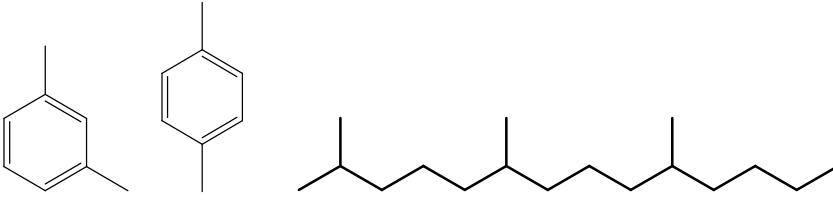
Table 5.5 **Products formed from the defunctionalisation and aromatisation of model compounds (280°C, 16h, 6 mg Pt/C per mg).**

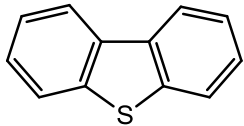
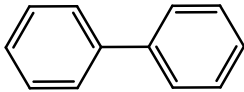
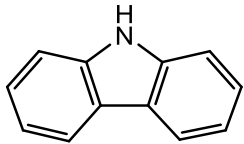
Model Compound	Major Products	Minor Products
<i>Cyclic alcohols, ketones and carboxylic acids</i>		
 2-Ethylcyclo-1-hexanol (0 %)	   Ethylbenzene (44 %) Benzofuran (23 %) 2-Ethylphenol (16 %)	Toluene (8%) Phenol (3 %) o-Methylphenol (2 %) o-Methylbenzofuran (1 %)
 1-Adamantanol (3 %)	  1-Chloroadamantane (66 %) Adamantane (30 %)	1,3-Chloroadamantane (<1 %)
 2-Methylcyclohexanone (0 %)	  o-Cresol (88 %) Phenol (5 %)	Benzofuran (<1 %) m/p-Cresol (<1 %) o-Methoxyphenol (<1 %)

 <p><i>trans</i>-4-Pentylcyclohexanoic acid (66 %)</p>	 <p>Pentylbenzene (18 %)</p>  <p>Pentylbenzoic acid (11 %)</p>	<p>1-Methylnaphthalene (2 %) Naphthalene (1 %) Toluene (<1 %) Ethylbenzene (<1 %) Butylbenzene (<1 %) Pentylcyclohexane (<1 %)</p>
 <p>Abietic acid (unknown %)</p>	 <p>Retene (51 %)</p>  <p>Methylretenes (17 %)</p>	<p>1,2,3-Trimethylbenzene (6 %) 1,7-Dimethylphenanthrene (5 %) Toluene (4 %) <i>m/p</i>-Xylene (5 %) 1,2,5-Trimethylnaphthalene (1 %)</p>
<p>Phenols and aromatic ketones, esters, and carboxylic acids</p>		
 <p>2,4,6-Trimethylbenzoic acid (0 %)</p>	 <p>1,3,5-Trimethylbenzene (89 %)</p>  <p>Toluene (2 %)</p>	<p><i>m/p</i>-Xylene (2 %)</p>

 <p>4-Isopropylphenol (74 %)</p>	 <p>4-Ethylphenol (11 %)</p>  <p><i>p</i>-Cresol (7 %)</p>	<p>Isopropylbenzene (<1 %)</p>
 <p>2,6-Dimethoxytoluene (7 %)</p>	 <p>Toluene (33 %)</p>  <p>2-Methoxytoluene (32 %)</p>  <p><i>o</i>-Cresol (14 %)</p>	<p>Benzofuran (8 %)</p> <p>3-Methoxy-2-methylphenol (3 %)</p> <p>Phenol (2 %)</p> <p>2-Methyl-benzofuran (1 %)</p>
 <p>9-Fluorenone (99 %)</p>	 <p>Biphenyl (1 %)</p>	

Straight chain primary alcohols, aldehydes, carboxylic acids and esters		
 Tetradecanal (0 %)	 Tridecane (32 %)  C7-Alkylbenzenes (48 %)	Tetradecanoic acid (11 %) 2-Methylbiphenyl (5 %) Toluene (1 %) Ethylbenzene (< 1 %) o-Xylene (< 1 %) C7-Alkenylbenzenes (< 1 %)
 Tetradecanoic acid (96 %)	 C7-Alkylbenzenes (1 %)  2-Methylbiphenyl (1 %)	Toluene (1 %) Ethylbenzene (< 1 %)
 Oleic acid (unknown %)	 Benzene (46 %)  C11-Alkylbenzenes (19 %)  C5-Alkylbiphenyls (12 %)	n-Hexadecanoic acid (5 %) C5-Alkylbenzenes (4 %) C3-Alkylbenzenes (2 %) Toluene (3 %) Ethylbenzene (1 %) o-Xylene (1 %)

 <p>Methyl hexadecanoate (1 %)</p>	 <p><i>n</i>-Hexadecanoic acid (95 %)</p>	<p>C9-Alkylbenzenes (2 %) C2-Alkylbiphenyls (2 %)</p>
<p><i>Straight chain secondary, and branched chain primary alcohols</i></p>		
 <p>2-Dodecanol (0 %)</p>	 <p>2-Dodecanone (96 %)</p>	<p>Toluene (1 %) Cyclised and aromatised aldehyde* (2 %)</p>
 <p>Phytanol (unknown %)</p>	 <p><i>m/p</i>-xylene (34 %) 6,10,14-trimethylpentadecanone (15 %)</p>	<p>Toluene (4 %) C13-Alkylbenzenes (11 %) Pristane (10 %) C12-Alkylbenzenes (4 %) C7-Alkylbenzene (6 %) C9-Alkylbenzene (1.5 %) 1,6-Dimethylnaphthalene (1 %) 1,3,6-Trimethylnaphthalene (2 %) C5-Alkyl-naphthalene (2 %)</p>

<i>Heterocyclic sulfur and nitrogen containing compounds</i>		
 Dibenzothiophene (89 %)	 Biphenyl (11 %)	
 Carbazole (100 %)	None	

5.5.1.1.7 Model compound mixture reaction products

Defunctionalisation of a model compound mixture at optimum conditions (280°C, 16 hours, ~6 mg pt/C per mg model compound) produced a range of hydrocarbons (Fig. 5.1). Percentage yields that were calculated for hydrocarbon reaction products showed that hydrocarbons are formed with reasonable yields in most instances.

A few minor differences were evident in the reaction products formed from individual model compound and model compound mixture experiments. This included some minor alkylation products in the defunctionalisation of the model compound mixture (alkylfluorenes 0.2 % and alkylcarbazoles 0.1 %; Fig. 5.1a). 9-Fluorenone was also depleted from the model compound mixture after defunctionalisation with its likely reaction product fluorene formed (7 % yield), whereas the defunctionalisation of 9-fluorenone alone resulted in negligible defunctionalisation (less than 1 % biphenyl formed). This suggests that dehydrogenation experiments carried out using a mixture of compounds (with a range of polar functionalities and carbon skeletons) may result in enhanced defunctionalisation to some compound types.

Some high molecular weight reaction products were also formed during model compound mixture defunctionalisation (Fig. 5.1b) that could not be identified. The formation of these compounds was likely a result of the high concentrations of a range of model compounds in the mixture reacting with each other to form larger compounds.

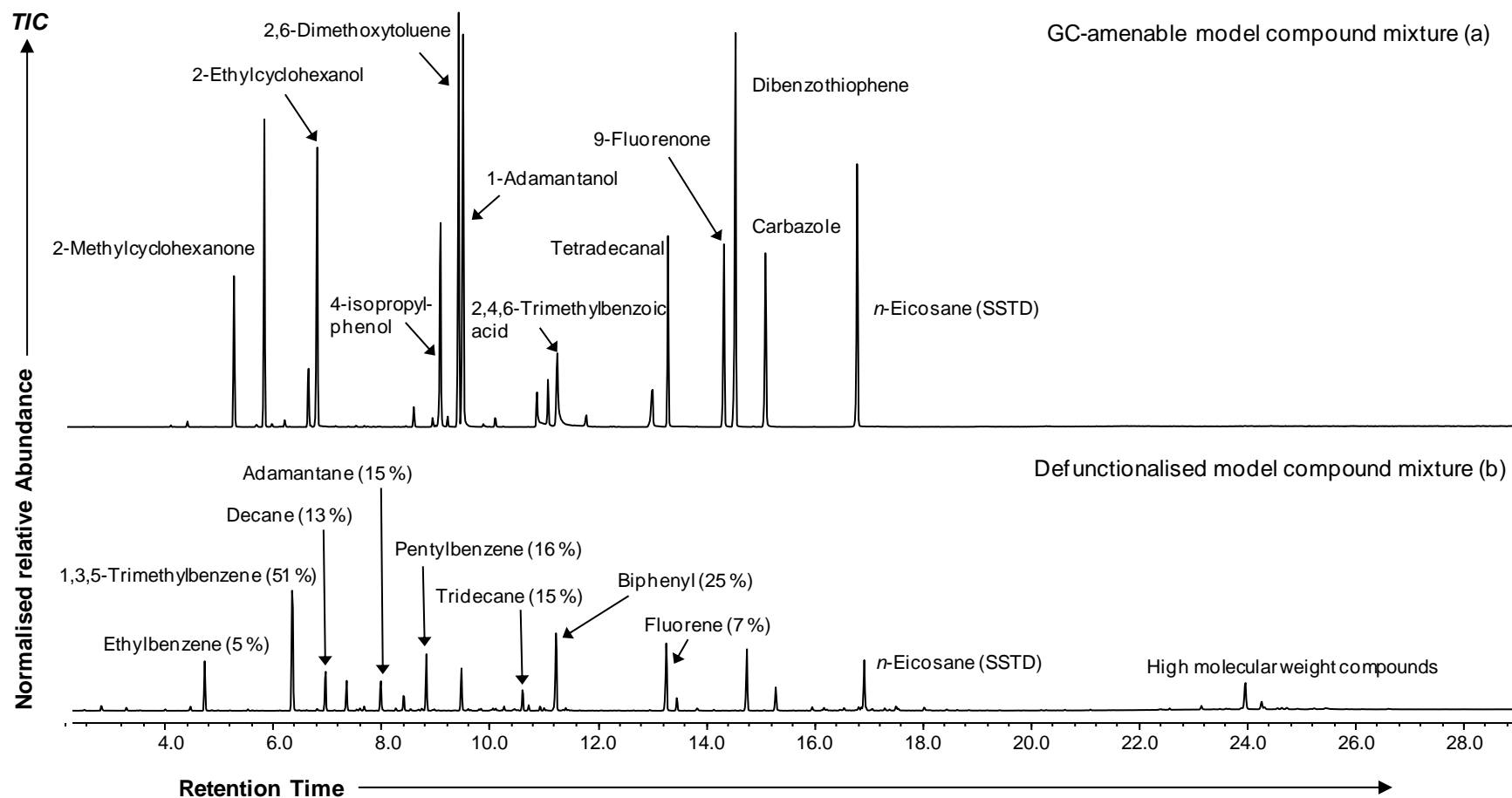


Figure 5.1 Total ion chromatograms (TIC) for a) the GC-amenable model compound mixture and b) major products (with % yields) formed from its defunctionalisation under optimal conditions (b) SSTD: surrogate standard

5.5.2 Defunctionalisation and aromatisation of polar UCMs

Polar UCMs from petroleum and NOM were used to evaluate the defunctionalisation technique. This included polar UCMs isolated from a weathered diesel, groundwater TPH extracts associated with weathered diesel contamination, and a TPH extract believed to contain primarily NOM.

5.5.2.1 Polar UCMs from petroleum

A weathered diesel polar fraction was used to assess the defunctionalisation technique. The weathered diesel was selected on the basis that it contained polar compounds that were present as a large UCM and individual compounds were difficult to identify (Fig. 5.2a). Defunctionalisation of the weathered diesel polar fraction (Fig. 5.2b) produced a significant number of abundant and resolved peaks that were identified as aliphatic and aromatic hydrocarbons, as well as heterocyclic polar compounds. The percentage recovery of extracted organics after defunctionalisation was 5 % based on the TIC response.

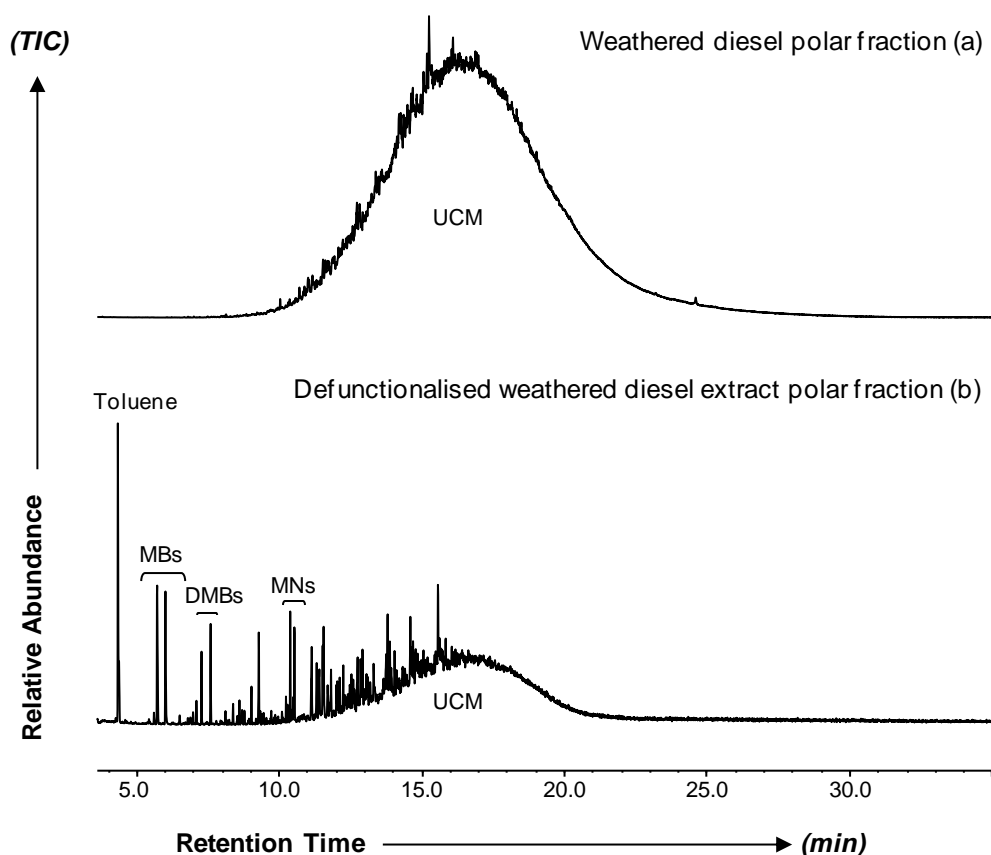


Figure 5.2 Total ion chromatograms (TICs) of a) weathered diesel polar fraction before and b) after defunctionalisation, analysed using GC-MS method 1. Chromatograms were background subtracted to remove column bleed. DMBs: dimethylbenzenes, TMBs: trimethylbenzenes, MNs: methylnaphthalenes: UCM: unresolved complex mixture.

Groundwater TPH extracts were selected from a range of samples obtained from the same site as the recovered weathered diesel. Selection was based on the samples containing large polar UCMs with no identifiable hydrocarbons, as well as one sample having no identifiable polar compounds. TICs for the TPH extracts from the groundwater samples (GW-1, Fig. 5.3a and GW-2, Fig. 5.4a) show the presence of large UCMs, with the GC profile for GW-1 showing some resolved peaks on the UCM and GW-2 having no resolved peaks. TPH concentrations for GW-1 and GW-2 were 34.4 and 17.7 mg/L respectively.

Separation of the groundwater (GW-1 and GW-2) TPH extracts into SAA and POL fractions showed that no aliphatic or aromatic components were present above detection limits (Fig. 5.3b, Fig. 5.4b, respectively), with the polar components comprising of the entire TPH (Fig. 5.3c, 5.4c, respectively). The polar POL fraction of GW-1 contained resolved peaks on the UCM that were identified as adamantanols (Fig. 5.3c), whereas the polar fraction of GW-2 consisted of a continuous UCM with no resolved or identifiable compounds (Fig. 5.4c). This suggests the TPH of GW-2 has experienced a greater extent of weathering.

Defunctionalisation of GW-1 (Fig. 5.3d) and GW-2 (Fig. 5.4d) polar fractions resulted in the formation of resolved peaks that were identified as aliphatic and aromatic hydrocarbons. Recoveries of organic material after defunctionalisation of polar fractions for GW-1 and GW-2 were 34.4 and 17.7 %, respectively based on their TIC responses.

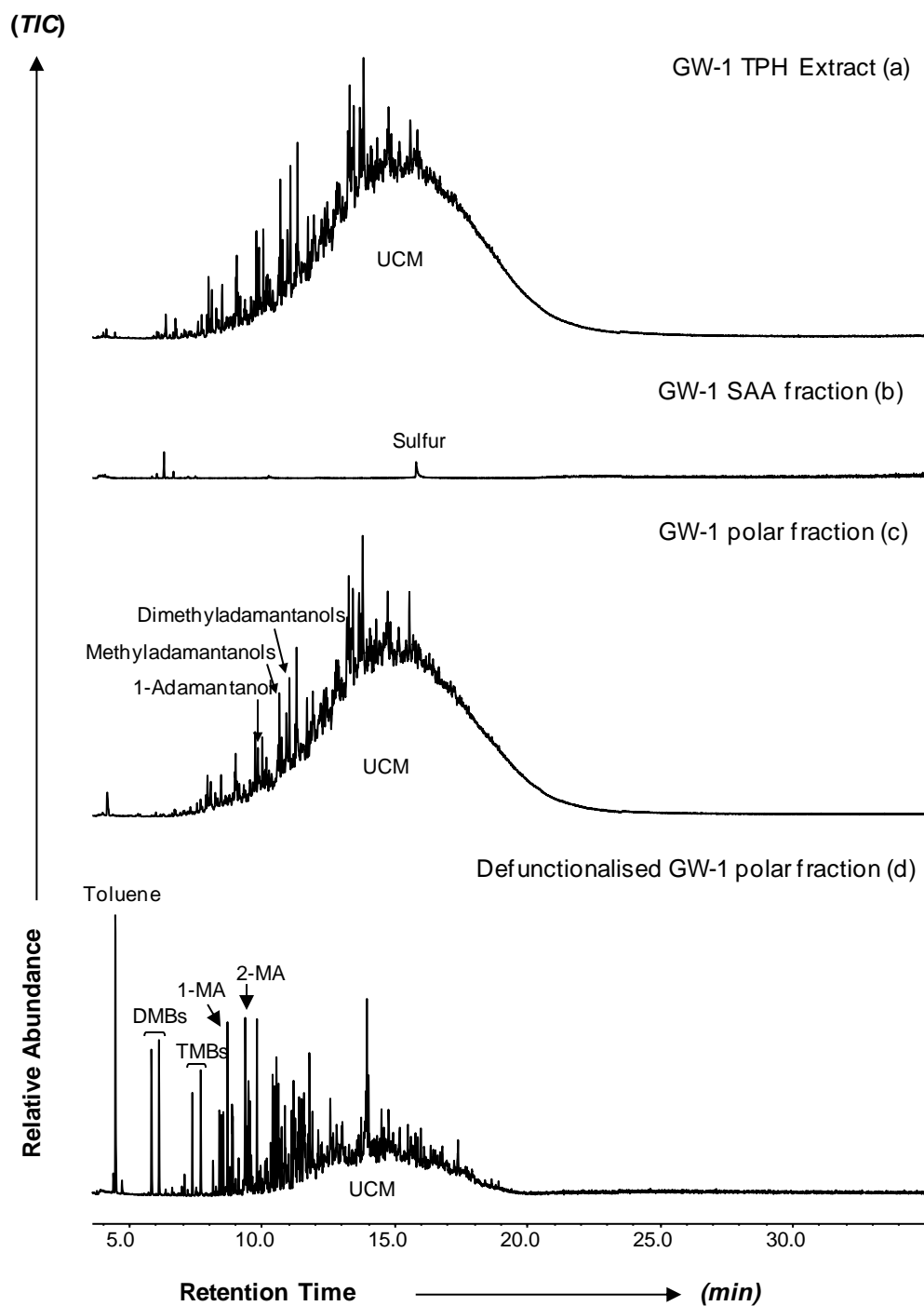


Figure 5.3 Total ion chromatograms (TIC) of a) GW-1 TPH extract, b) GW-1 TPH extract SAA fraction, c) GW-1 TPH extract polar fraction, and d) defunctionalised GW-1 TPH extract polar fraction, analysed using GC-MS method 1. Chromatograms background subtracted to remove column bleed. DMBs: dimethylbenzenes, TMBs: trimethylbenzenes, MA: methyladamantane: UCM: unresolved complex mixture.

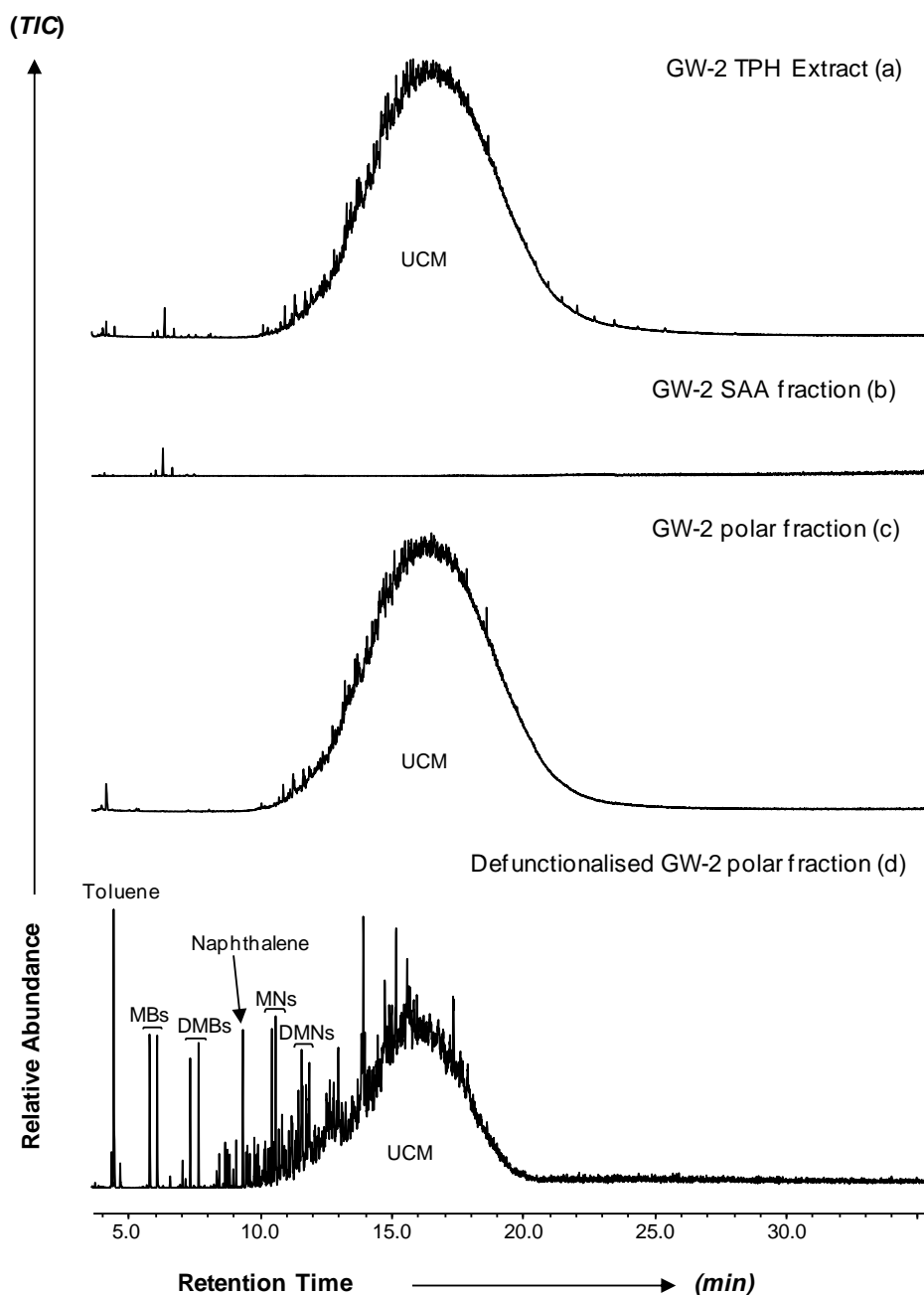


Figure 5.4 Total ion chromatograms (TIC) of a) GW-2 TPH extract, b) GW-2 TPH extract SAA fraction, c) GW-2 TPH extract polar fraction, and d) defunctionalised GW-2 TPH extract polar fraction analysed using GC-MS method 1. Chromatograms background subtracted to remove column bleed. DMBs: dimethylbenzenes, TMBs: trimethylbenzenes, MNs: methylnaphthalenes, DMNs: dimethylnaphthalenes UCM: unresolved complex mixture.

Compound class characterisation was carried out on the weathered diesel and groundwater defunctionalised polar fractions to determine the proportions of aliphatic, aromatic and polar components formed (Table 5.6). The aliphatic fraction contained the lowest amount of material (0 to 1 %), followed by the aromatic (1 % to 26 %) and polar fractions (73 % to 99 %).

Table 5.6 Proportions of aliphatic, aromatic and polar components formed in the defunctionalised weathered diesel polar fraction, and GW-1 and GW-2 TPH extract polar fractions

Compound class	Defunctionalised products		
	Weathered diesel	GW-1	GW-2
Aliphatic fraction	1 %	< 1 %	0 %
Aromatic fraction	26 %	4 %	1 %
Polar fraction	73 %	95 %	99 %

5.5.2.1.1 Major reaction product types

Aliphatic hydrocarbons

Adamantanes were abundant aliphatic reaction products formed from the defunctionalisation of the weathered diesel and groundwater polar fractions. The adamantanes identified included adamantane and a suite of C1-, C2-, C3- and C4-alkyladamantane isomers (Fig. 5.5). Precursors to the alkyladamantane reaction products are likely degradation products of alkyladamantanes (Mair *et al.*, 1959; Petrov *et al.*, 1974; Wingert, 1992). Distributions of the alkyladamantane isomers formed from the weathered diesel polar fraction and groundwater polar fractions were very similar.

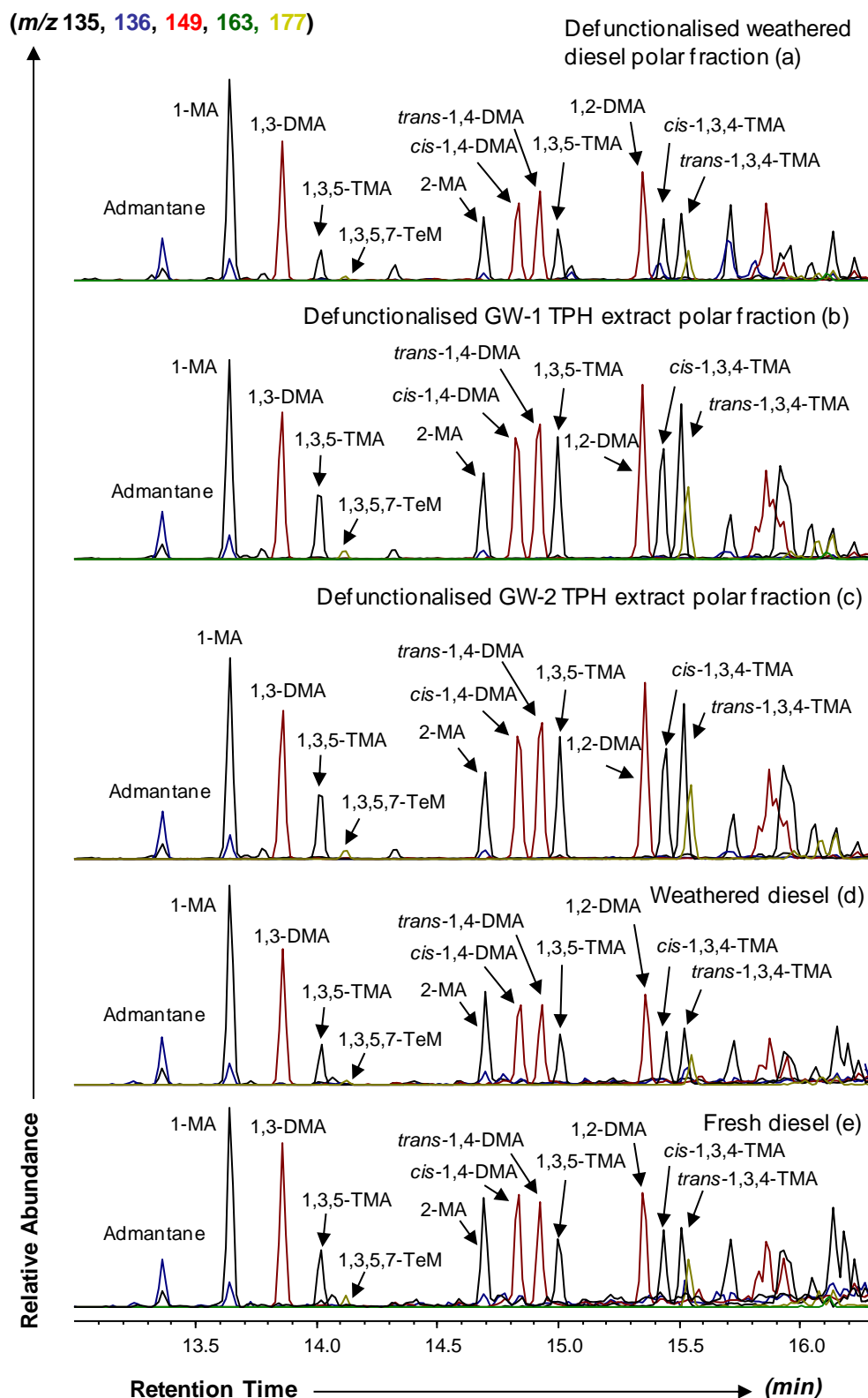


Figure 5.5 Partial ion chromatograms (*m/z* 135, 136, 149, 163, 177 and 191) showing adamantanes formed from the defunctionalisation of a) weathered diesel polar fraction, b) GW-1 TPH extract polar fraction, and c) GW-2 TPH extract polar fraction, and adamantanes present in d) weathered and e) fresh diesels analysed using GC-MS method 2. MA: methyladamantane, DMA: dimethyladamantane, TMA: trimethyladamantane, TeMA: tetramethyladamantane.

Comparison of the alkyladamantane reaction products with alkyladamantanes present in weathered (Fig. 5.5d) and fresh diesel (Fig. 5.5e) show a very similar distribution of isomers. This suggests the process of degradation (to form the polar compounds present in the UCM) and defunctionalisation (to form reaction products) may not have resulted in the loss of any alkyl groups. This may only occur where specific pathways of biodegradation have taken place, and therefore can be used to suggest the original compound types present in the UCM that formed the hydrocarbons in the reaction products. For example, adamantane biodegradation products formed from the oxidation of a methyl group (e.g. carboxylic acids) would undergo decarboxylation during defunctionalisation, and result in the loss of a methyl group from the original carbon skeleton. On the other hand, adamantane biodegradation products formed through hydroxylation of the cyclic ring (i.e. adamantanols) would defunctionalise while keeping the carbon skeleton intact. Therefore, alkyladamantanes with polar functional groups attached directly to the adamantane carbon skeleton may be precursors for the alkyladamantane reaction products. This is not surprising, as adamantanols were originally identified in the GW-1 TPH extract polar fraction prior to defunctionalisation, and their presence in water samples associated with weathered petroleum was described in detail in Chapter 4. However, the formation of alkyladamantanes as major reaction products of the GW-2 TPH is less expected, as the polar fraction originally contained no identifiable adamantanols. Therefore, the alkyladamantanes formed from GW-2 may be a result of enrichment of alkyladamantanols present below detection limits or other polar compounds containing the adamantane carbon skeleton, such as compounds containing more than one hydroxyl group (i.e. polyols).

The similar distributions of alkyladamantanes in the reaction products, and fresh and weathered diesels suggests there has been no significant depletion of specific alkyladamantane isomers. A previous study showed the ratio of methyladamantanes to adamantanes i.e. (1- and 2-methyladamantane/adamantane) increases in crude oil with an increased

level of biodegradation (Grice *et al.*, 2000; Wei *et al.*, 2007), and therefore a higher abundance of adamantane relative to methyladamantanes would be expected in reaction products (a result of the increased abundance of the adamantane biodegradation product in the polar UCM). However, this was not shown for the reaction products, suggesting the degradation has been non-specific.

Higher molecular weight diamondoids were also formed from the defunctionalisation of petroleum UCMs. These included compounds containing the diamantane and triamantane carbon skeleton (Fig. 5.6).

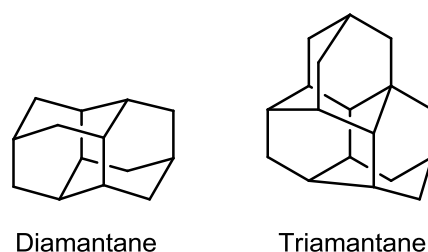


Figure 5.6 Structures of diamantane and triamantane

Diamantanes were clearly observed in the defunctionalised weathered diesel polar fraction (Fig. 5.7a) and groundwater TPH extracts (Fig. 5.7b,c). Similar to the adamantanes, the di- and triamantanes are also likely formed from the defunctionalisation of polar compounds with intact diamondoid carbon skeletons. Triamantane was present in very low relative abundances in the defunctionalised UCMs from petroleum (Fig. 5.8a) and GW-1 TPH extract (Fig. 5.8b). A higher abundance of triamantane and methyltriamantanes were present in the defunctionalised GW-2 polar fraction and may be a result of the higher extent of biodegradation of GW-2 compared with GW-1. Triamantanes were not identified in fresh and weathered diesels, and as a result the distributions of triamantanes present in fresh and weathered diesel could not be compared to those formed from defunctionalisation.

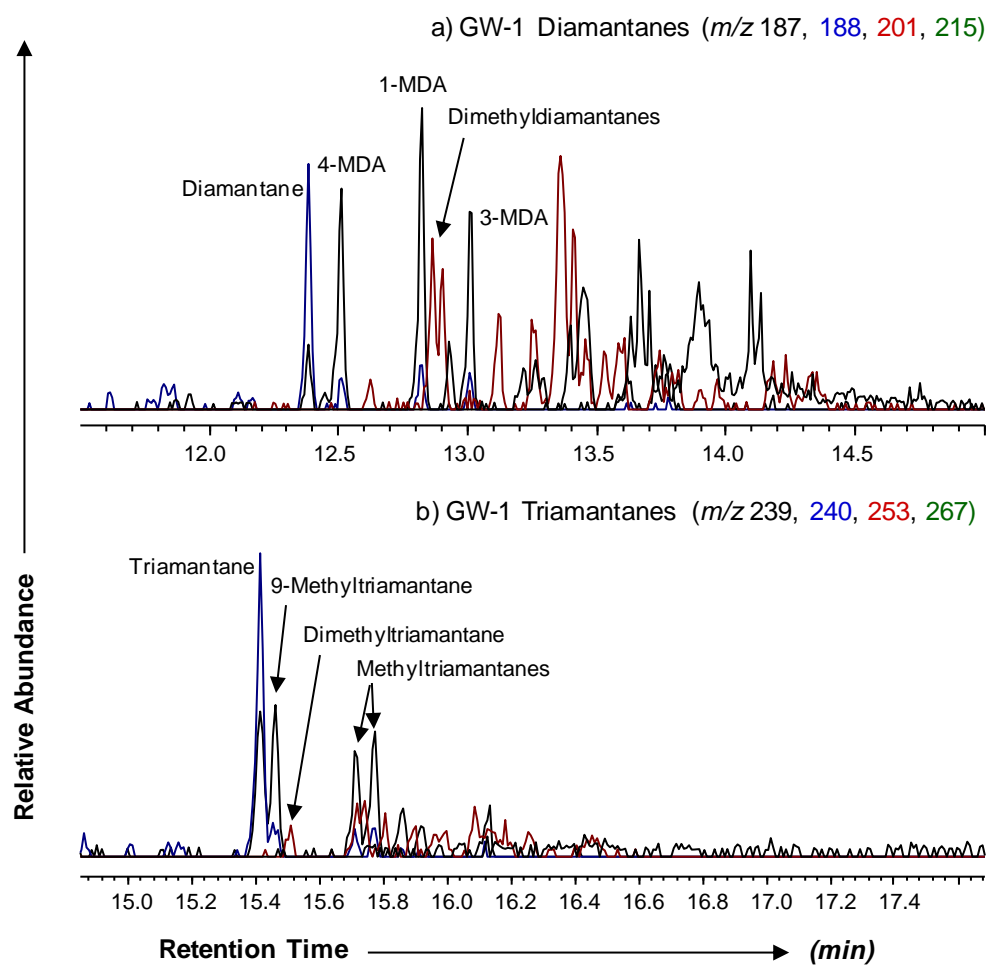


Figure 5.7 Partial ion chromatogram (m/z 135, 136, 149, 163, 177 and 191) showing diamantanes formed from the defunctionalisation of a) weathered diesel polar fraction and b) GW-1 TPH extract polar fraction, c) GW-2 TPH extract polar fraction, and diamantanes present in d) weathered and e) fresh diesels analysed using GC-MS method 2. MDA: methyldiamantane, *: tentatively identified,

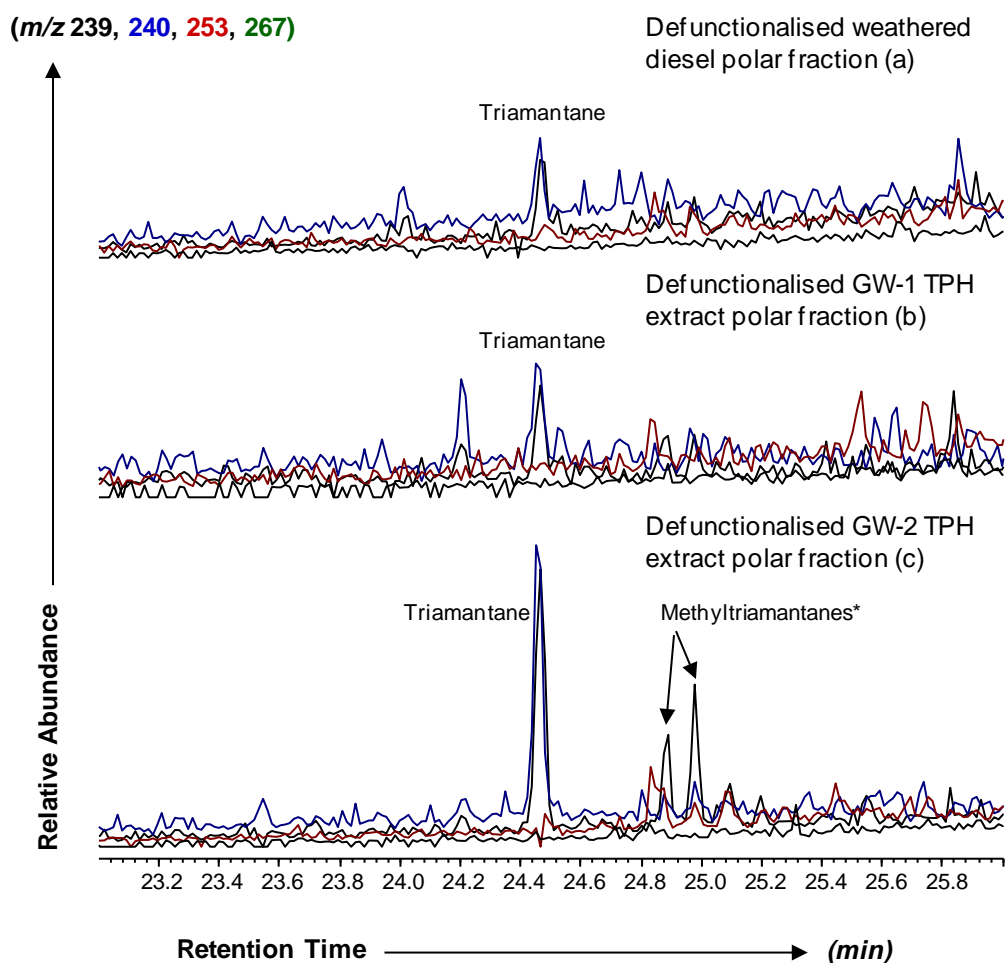


Figure 5.8 Partial ion chromatograms (m/z 239, 240, 253, 267) showing triamantanes formed from defunctionalisation of a) weathered diesel polar fraction, b) GW-1 TPH extract polar fraction and c) GW-2 TPH extract polar fraction analysed using GC-MS method 2. *:tentatively identified,

Aromatic hydrocarbons

A range of aromatic hydrocarbons were identified in the defunctionalised polar UCMs from petroleum. This included alkylbenzenes, alkyl-naphthalenes, diphenylmethane and alkylbiphenyls. Alkylbenzenes and alkyl-naphthalenes were the most abundant of these reaction products and can be clearly observed in the TPH chromatograms (Fig. 5.2b, 5.3b and 5.4b). The aromatic hydrocarbons formed in the defunctionalisation reactions have a range of possible sources in the polar fractions. Carbon skeletons of polar compounds that can form aromatic hydrocarbons upon defunctionalisation include those with aromatic, cyclic (via aromatisation), and straight and branched chain (via

cyclisation and aromatisation) structures. None of these precursors were identified in the polar fractions prior to defunctionalisation.

Alkylbenzenes

Alkylbenzenes formed from the defunctionalisation of the weathered diesel polar fraction and groundwater TPH polar fractions included C2-C4 alkylated homologs (Fig. 5.9-11). Comparison of C2-, C3- and C4-alkylbenzenes distributions formed from the defunctionalisation of polar UCMs from petroleum with the distributions present in weathered and fresh diesels (Fig. 5.9-11) shows that all possible methylated isomers have formed. The presence of a range of alkylbenzene isomers with no obvious dominance of *ortho*-substituted alkylbenzene isomers compared to fresh diesel suggests that the cyclisation of straight chain primary substituted polar compounds has not been a dominant process. This is not surprising considering that long chain carboxylic acids are likely to be present only as short lived biodegradation intermediates of *n*-alkanes (Watson *et al.*, 2002; Wentzel *et al.*, 2007), which are the most susceptible compound type to biodegradation in petroleum (Kaplan *et al.*, 1996). Alkylbenzenes containing branched alkyl groups were also not identified as major products, also indicating that the branched chain primary substituted polar compounds (i.e. degradation products of functionalised isoprenoids) were not dominant carbon skeleton types in the polar UCM.

The distributions of C2-alkylbenzenes formed from the defunctionalisation of polar UCMs from petroleum were similar to those present in fresh diesel, but differed significantly to those present in the weathered diesel (Fig. 5.9). The limited number of C2-alkylbenzene isomers makes it difficult to assess their likely sources in the defunctionalisation products. The distributions of C3- and C4-alkylbenzenes formed from the defunctionalisation of polar UCMs from petroleum (Fig. 5.10a,b,c and 5.11a,b,c) differed from those present in a fresh diesel (Fig. 5.10e and 5.11a,b,c) and showed some similar features to the distributions present in a weathered diesel (Fig. 5.10d and 5.11d). This includes a higher abundance of isomers that are relatively resistant to

biodegradation (1,2,3-trimethylbenzene and 1,2,3,4-tetramethylbenzene; George *et al.* 2002).

The C4-alkylbenzenes in the weathered samples contained higher relative abundances of the ethyl-containing isomers (ethyldimethylbenzenes) compared to C3-alkylbenzenes. Although the distributions of tri- and tetramethylbenzenes in the reaction products are similar to the weathered diesel, likely degradation products of the di-, tri- and tetramethylbenzenes (alkylphenols) were not identified in the water equilibrated with weathered diesel in Chapter 4. Therefore possible precursors for the C2-, C3- and C4-alkylbenzenes reaction products include catechols, benzoic acids, cyclic alcohols, cyclic ketones and cyclic carboxylic acids.

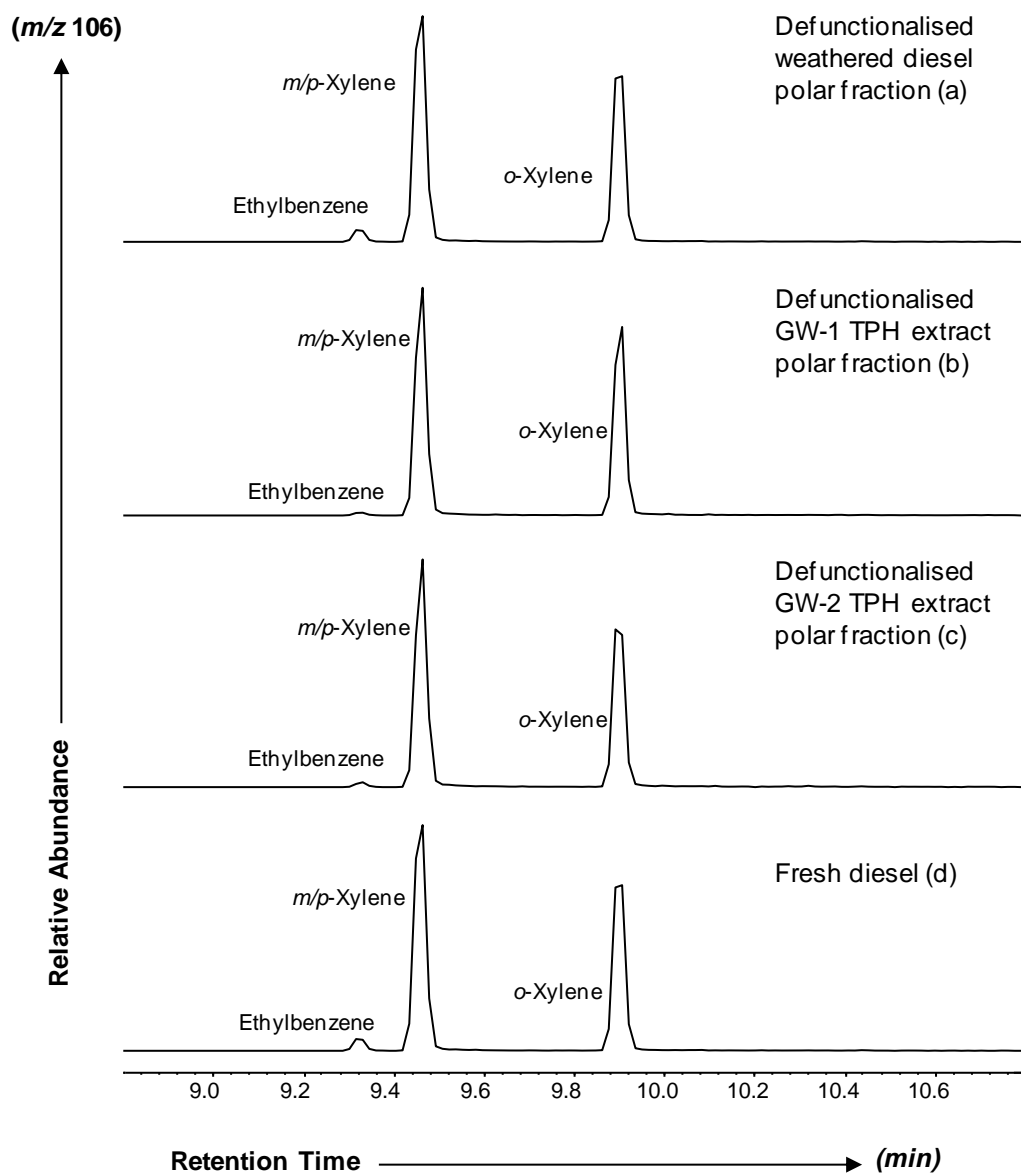


Figure 5.9

Figure 5.9 Partial ion chromatograms (m/z 106) showing C2-alkylbenzenes formed from defunctionalisation of a) weathered diesel polar fraction, b) GW-1 TPH extract polar fraction, and c) GW-2 TPH extract polar fraction, and C2-alkylbenzenes present in d) weathered, and e) fresh diesels analysed using GC-MS method 2.

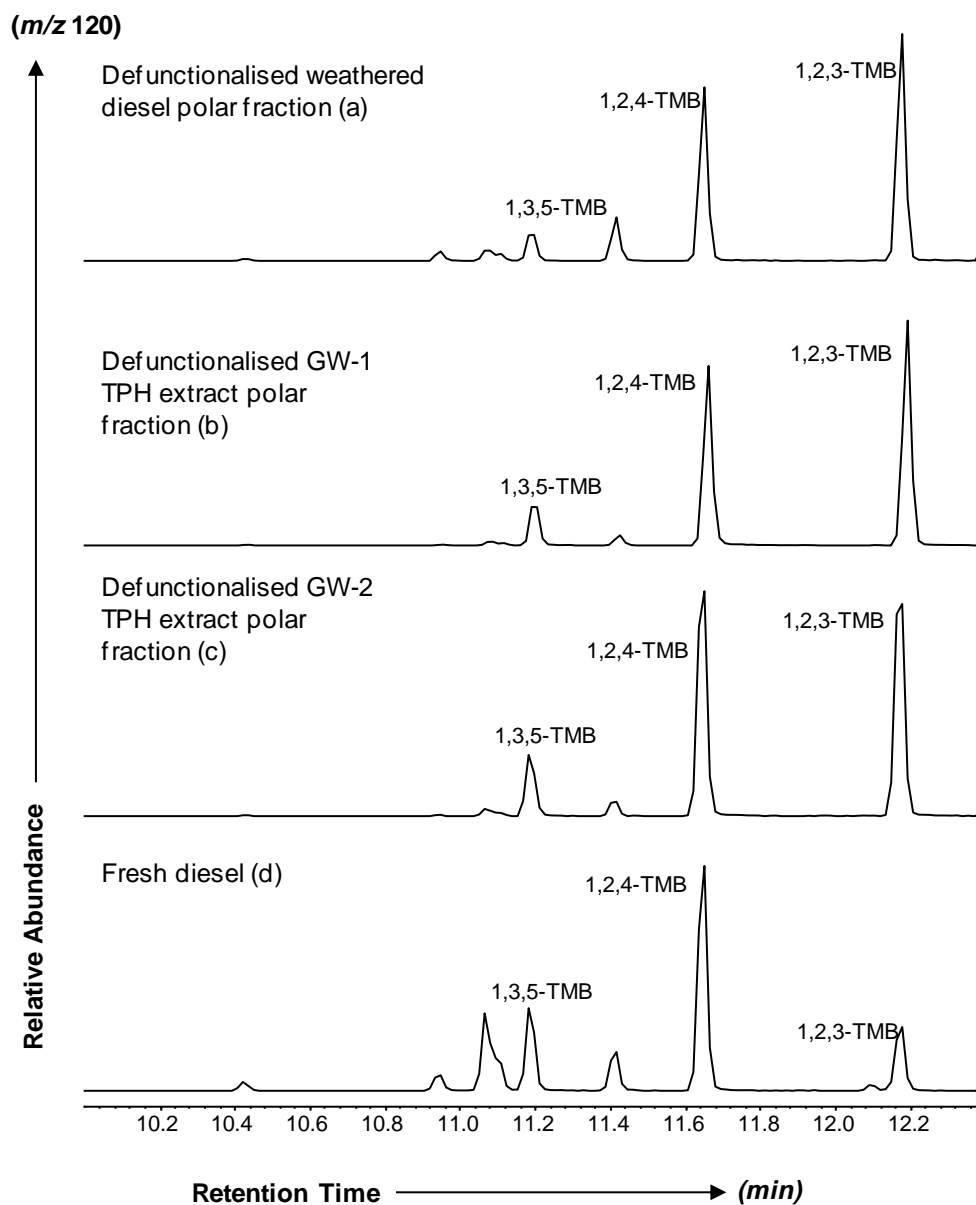


Figure 5.10 Partial ion chromatograms (m/z 120) showing C3-alkylbenzenes formed from defunctionalisation of a) weathered diesel polar fraction, b) GW-1 TPH extract polar fraction, c) GW-2 TPH extract polar fraction, and C3-alkylbenzenes present in d) weathered and e) fresh diesels analysed using GC-MS method 2. TMB: trimethylbenzene.

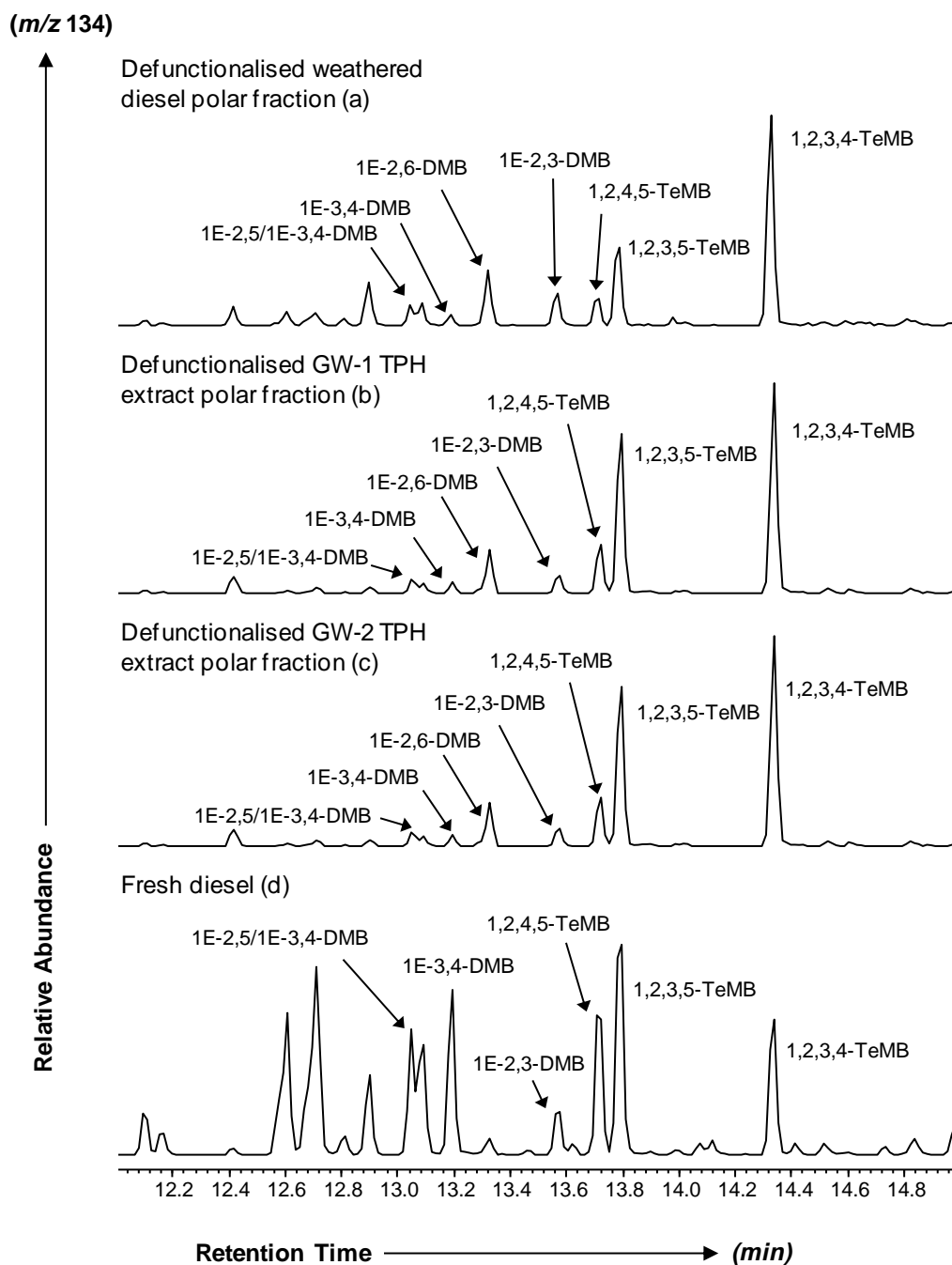


Figure 5.11 Partial ion chromatograms (m/z 134) showing C4-alkylbenzenes formed from defunctionalisation of a) weathered diesel polar fraction, b) GW-1 TPH extract polar fraction, c) GW-2 TPH extract polar fraction, and C4-alkylbenzenes present in d) weathered and e) fresh diesels analysed using GC-MS method 2. E: ethyl, B: benzene, DM: dimethyl, TeMB; tetramethylbenzene.

Alkylnaphthalenes

Alkylnaphthalenes were formed from the defunctionalisation of all petroleum polar UCMs (Fig. 5.12,13 and 14) and formed all the di-, tri- and tetramethylnaphthalenes present in the fresh and weathered diesels. The distribution of alkylnaphthalenes formed from defunctionalisation had higher relative abundances of 1,2-dimethylnaphthalene, 1,2,5-trimethylnaphthalene and 1,2,5,6-/1,2,3,5-tetramethylnaphthalene. The higher relative abundance of these alkylnaphthalenes in the reaction products is likely a result of polar compound sources containing this configuration. These configurations include drimane (Fig. 5.15) biodegradation products, with drimanes being common bicyclic constituents of petroleum (Alexander *et al.*, 1984). Additional polar compound types that could form the alkylnaphthalenes include polar compounds containing bicyclic carbon skeletons, such as naphthalene, tetrahydronaphthalene and decahydronaphthalene moieties identified in Chapter 4 (i.e. naphthoic acids, tetrahydronaphthoic acids, tetralones and decalones).

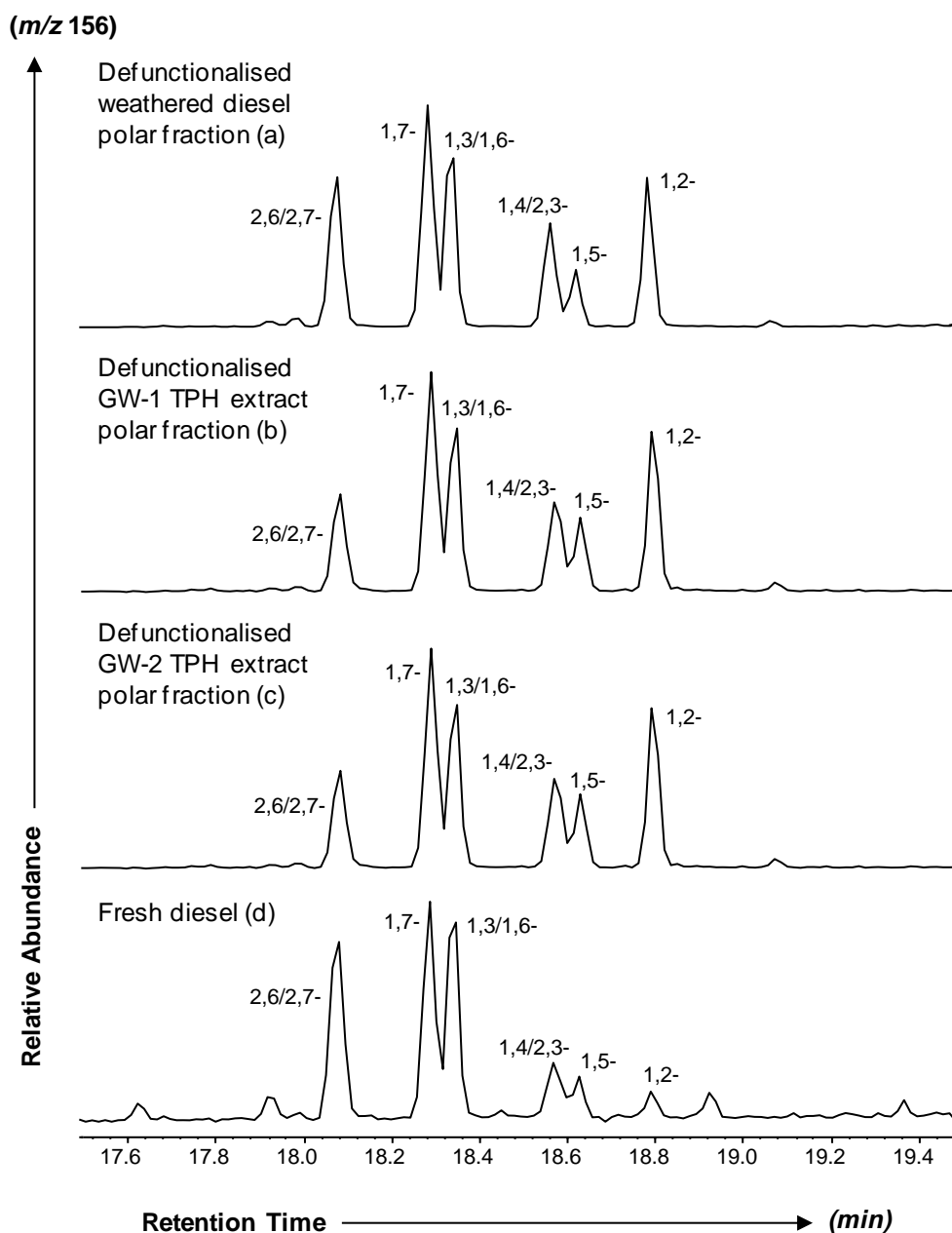


Figure 5.12 Partial ion chromatograms (m/z 156) showing dimethylnaphthalenes formed from defunctionalisation of a) weathered diesel polar fraction, b) GW-1 TPH extract polar fraction, c) GW-2 TPH extract polar fraction, and C2-alkylnaphthalenes present in d) weathered and e) fresh diesels analysed using GC-MS method 2.

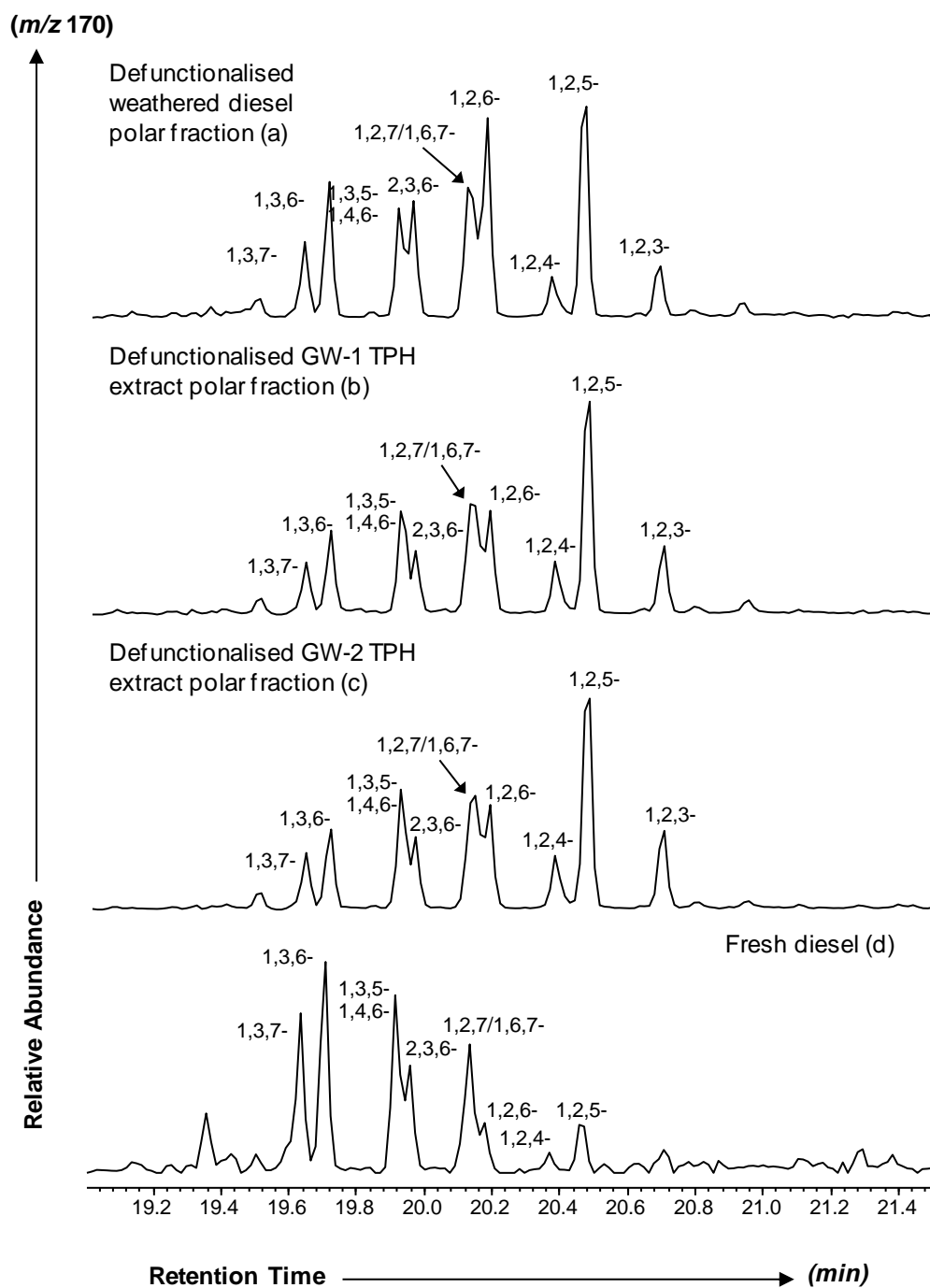


Figure 5.13 Partial ion chromatograms (m/z 170) showing trimethylnaphthalenes formed from the defunctionalisation of a) weathered diesel polar fraction, b) GW-1 TPH extract polar fraction, c) GW-2 TPH extract polar fraction, and trimethylnaphthalenes present in d) weathered and e) fresh diesels analysed using GC-MS method 2.

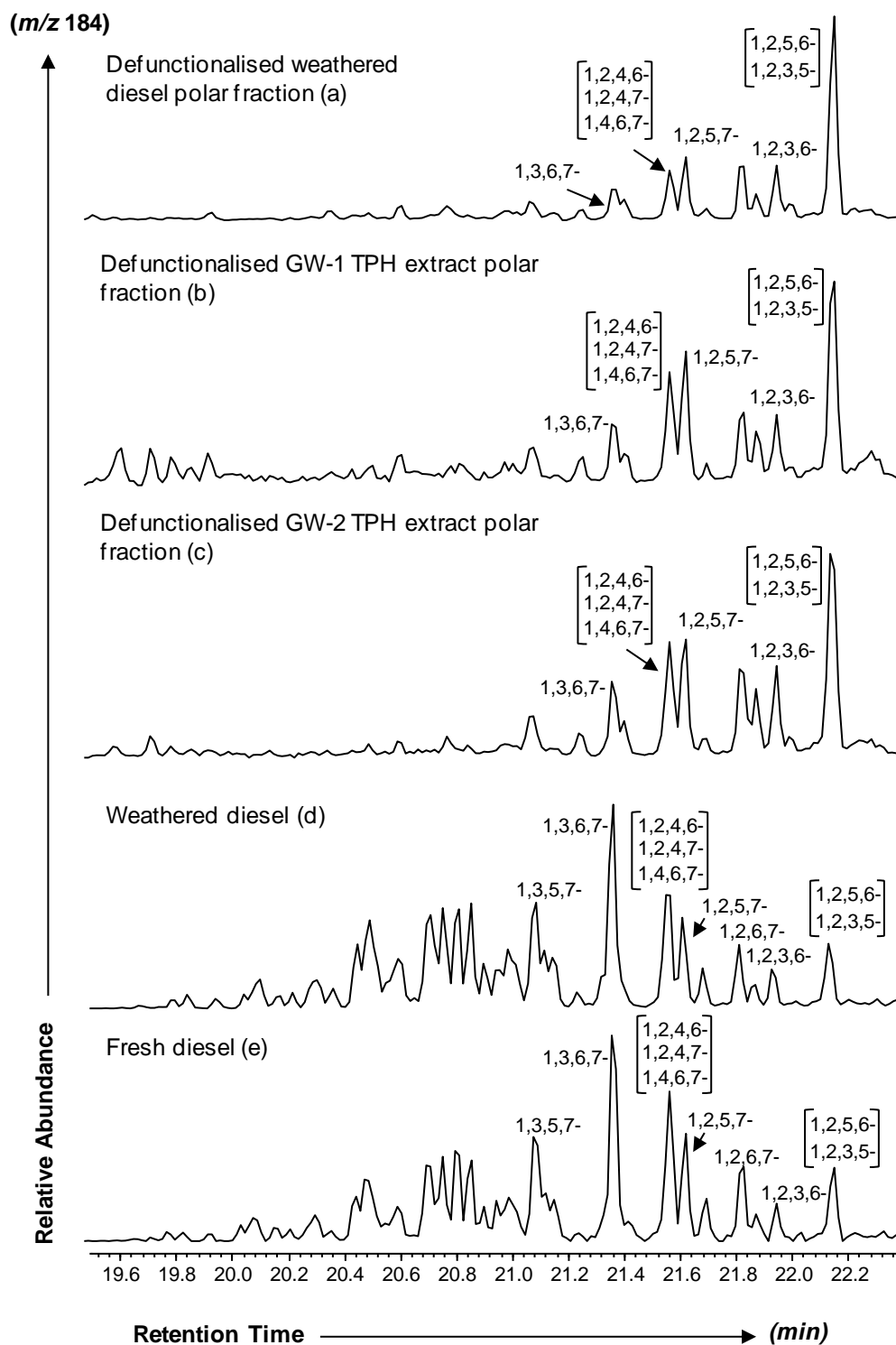


Figure 5.14 Partial ion chromatograms (m/z 184) showing tetramethylnaphthalenes formed from the defunctionalisation of a) weathered diesel polar fraction, b) GW-1 TPH extract polar fraction, c) GW-2 TPH extract polar fraction, and tetramethylnaphthalenes present in d) weathered and e) fresh diesels analysed using GC-MS method 2.

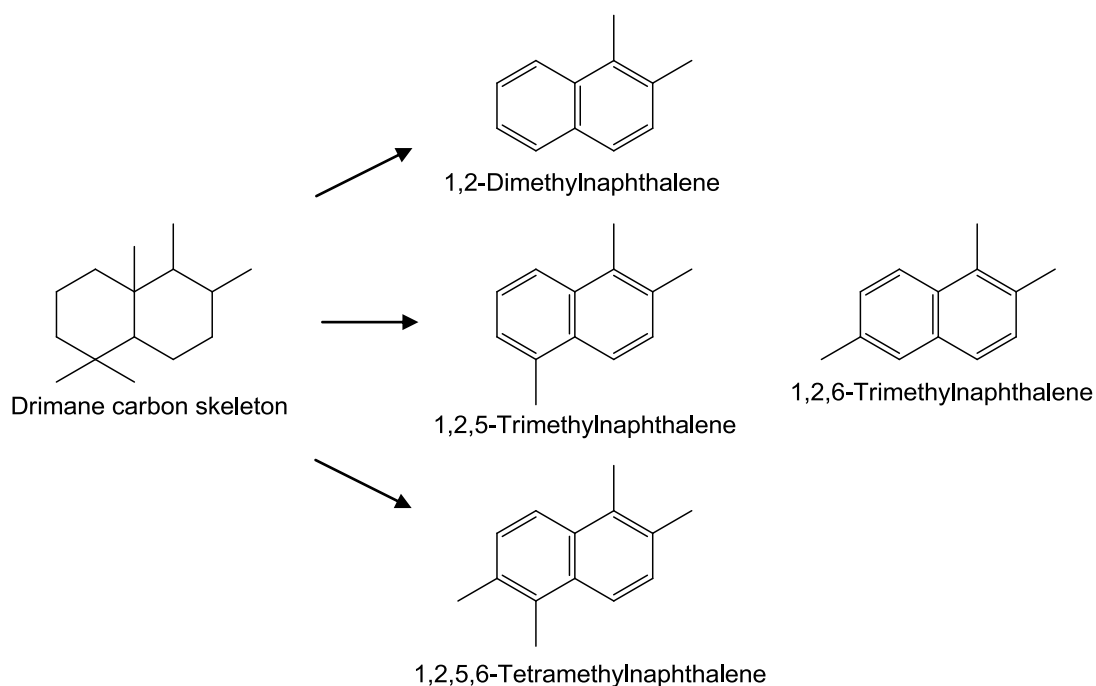


Figure 5.15 Proposed aromatisation of the drimane carbon skeleton to form a range methylated naphthalene isomers (excludes shift of the axial methyl group).

Diphenylmethane and alkylbiphenyls

Alkyldiphenylmethane and alkylbiphenyls were formed from the defunctionalisation of all polar UCMs from petroleum-derived sources (Fig. 5.16 and 17). This included a range of methylbiphenyls, dimethylbiphenyls, and methyldiphenylmethanes that are present in fresh and weathered diesels. 2-Methylbiphenyl, diphenylmethane and 2,2'-dimethylbiphenyl have higher relative abundances in the reaction products compared to fresh and weathered diesel, suggesting they have possibly formed through the cyclisation of straight chain primary substituted polar compounds. However, the absence of other cyclisation products (dominant *ortho*-substituted alkylbenzene isomers) of straight chain primary substituted polar compounds suggests this is not likely. As a result, the biphenyl, diphenylmethane reaction products are more likely to have formed from the defunctionalisation of polar compounds with the same carbon skeleton (e.g. biphenylacetic and diphenylacetic acids identified in groundwater; Chapter 4). However, the distributions of these reaction products were similar for the weathered diesel

polar fraction and the groundwater TPH extracts, and the polar compounds biphenylacetic and diphenylacetic acids were only identified in groundwater and not the weathered diesel in Chapter 4. As a result, other cyclic polar compounds containing the same carbon skeletons are the likely precursors (e.g. polyfunctional compounds).

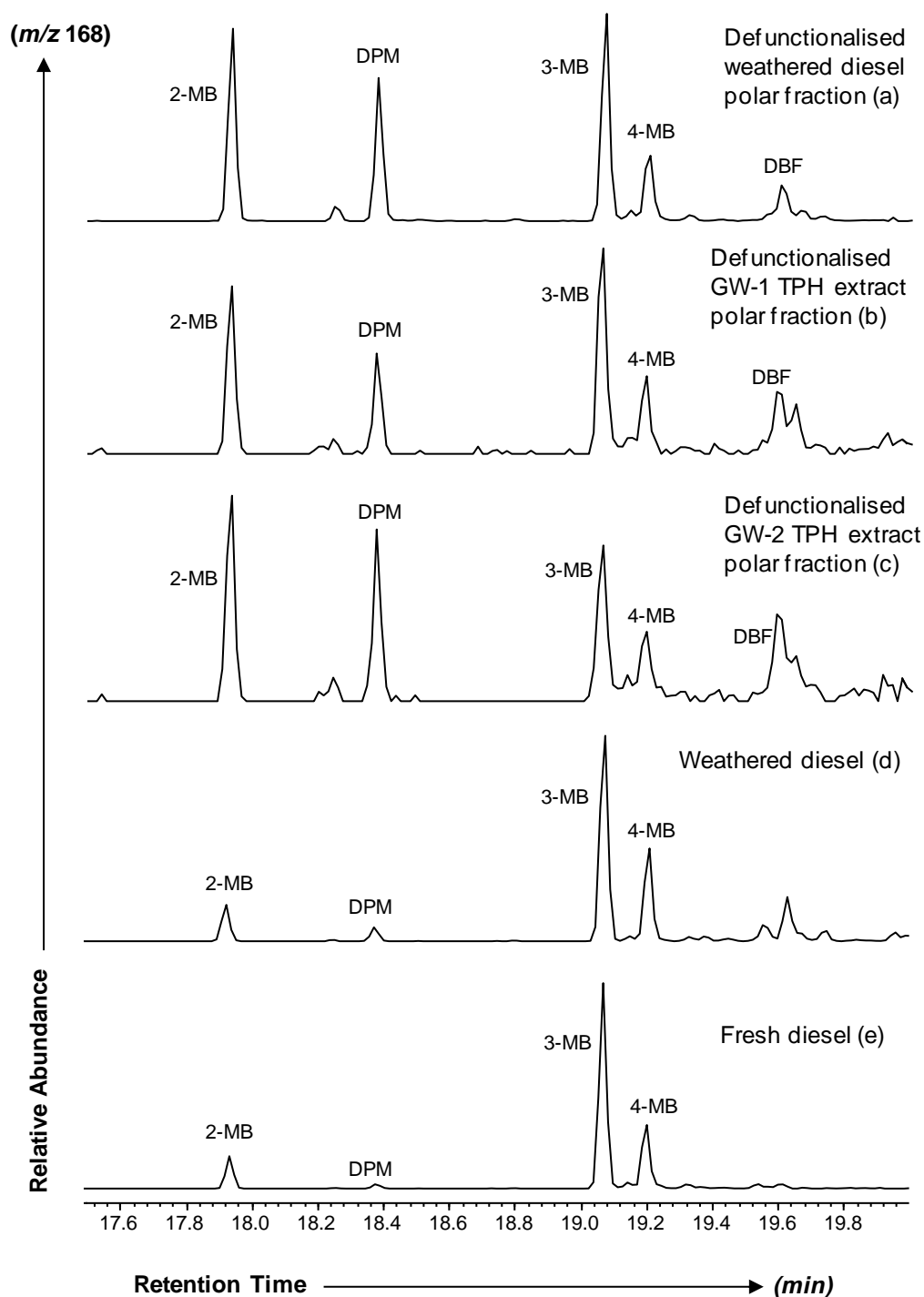


Figure 5.16 Partial ion chromatograms (m/z 168) showing diphenylmethane, dibenzofuran and methylbiphenyls formed from the defunctionalisation of a) weathered diesel polar fraction, b) GW-1 TPH extract polar fractions, c) GW-2 TPH extract polar fractions, and diphenylmethane and methylbiphenyls present in d) weathered and e) fresh diesels analysed using GC-MS method 2. DPM: diphenylmethane, DBF: dibenzofuran, MB: methylbiphenyl.

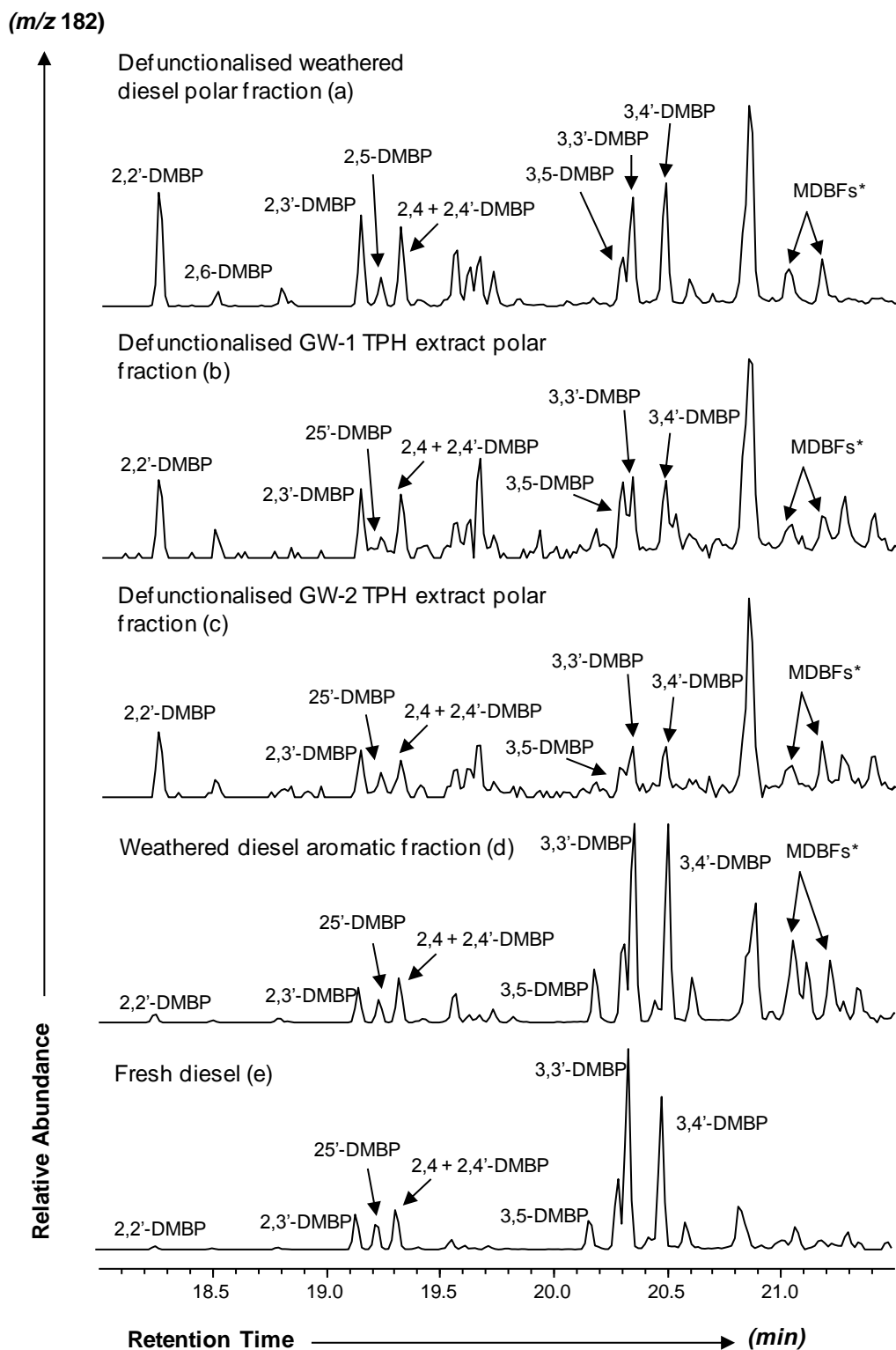


Figure 5.17 Partial ion chromatograms (m/z 182) showing dimethylbiphenyls formed from defunctionalisation of a) weathered diesel polar fraction and b) GW-1 TPH extract polar fractions, c) GW-2 TPH extract polar fractions, and dimethylbiphenyls present in d) weathered and e) fresh diesels analysed using GC-MS method 2. DMBP: dimethylbiphenyl, MDBFs: methyl dibenzofurans* (tentatively identified).

Polar compounds

Heterocyclic oxygen containing polar compounds (methyldibenzofurans) were present in all defunctionalised polar fractions from petroleum, though heterocyclic sulfur containing polar compounds (alkylbenzothiophenes) were only present in the defunctionalised weathered diesel polar fraction.

Methyldibenzofurans were originally present in the weathered diesel, though they were not present in the polar fraction (eluting in the aromatic fraction due to their relatively lower polarities). Therefore methyldibenzofurans present in the reaction products (Fig. 5.17a,b,c) have formed from defunctionalisation, likely from polar compounds containing the methyldibenzofuran carbon skeleton. These polar compounds include biodegradation products of methyldibenzofurans (hydroxylation and carboxylation products) and dimethyldibenzofurans (oxidised at the methyl group). They may have also formed from hydroxylated dimethylbiphenyls with specific configurations via dehydrocyclisation mechanisms (Fig. 5.18) as similarly shown by the formation of benzofuran from the model compound 2-ethylcyclohexanol.

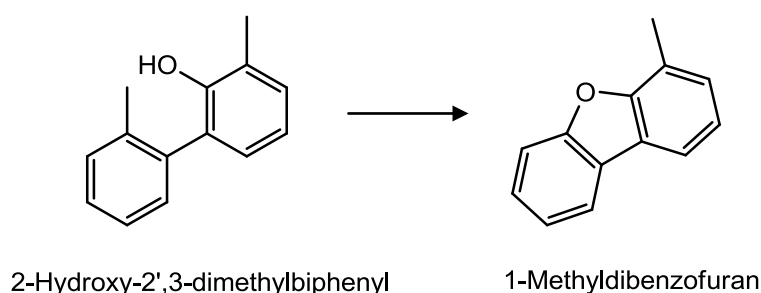


Figure 5.18 **Proposed dehydrocyclisation of 2-hydroxy-2',3-dimethylbiphenyl to form 1-methyldibenzofuran**

Alkylbenzothiophenes were only present in the defunctionalised weathered diesel polar fraction. Methylbenzothiophene (Fig. 5.19) and C2-alkylbenzothiophene (Fig. 5.20) isomer configurations could not be assigned, though a range of isomers are shown (one peak eliminated as a C2-alkylbenzothiophene due to its significantly different mass spectrum). These compounds were not present in the fresh and weathered diesel, so the distributions could not be compared. Possible polar compounds that can defunctionalise to form these reaction products include biodegradation products of methylbenzothiophenes and C2-alkylbenzothiophenes, as well as benzothiophenes oxidised at the sulfur atom (i.e. sulfanones and sulfoxides). A sulfanone was identified in the water phase of a weathered diesel equilibration experiment in Chapter 4, and therefore sulfanones may be possible parent compounds.

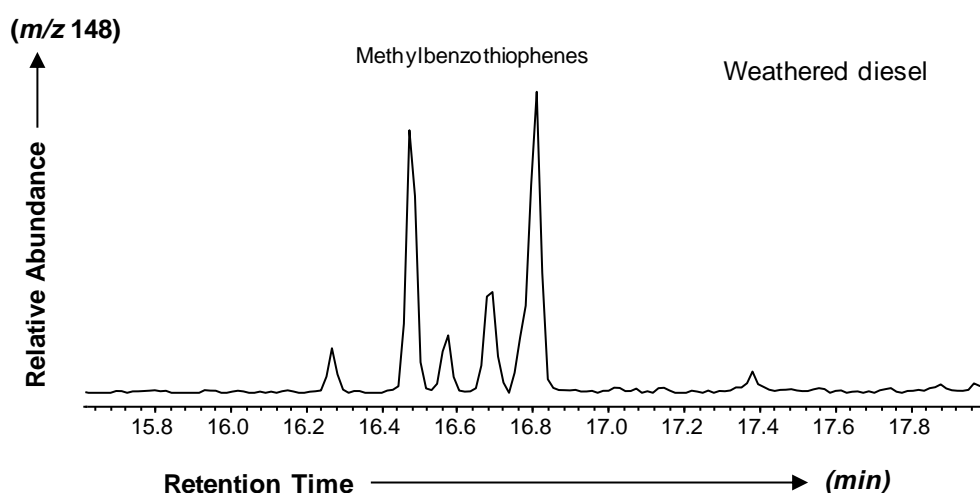


Figure 5.19 Partial ion chromatogram showing methylbenzothiophenes formed from defunctionalisation of the weathered diesel polar fraction analysed using GC-MS method 2.

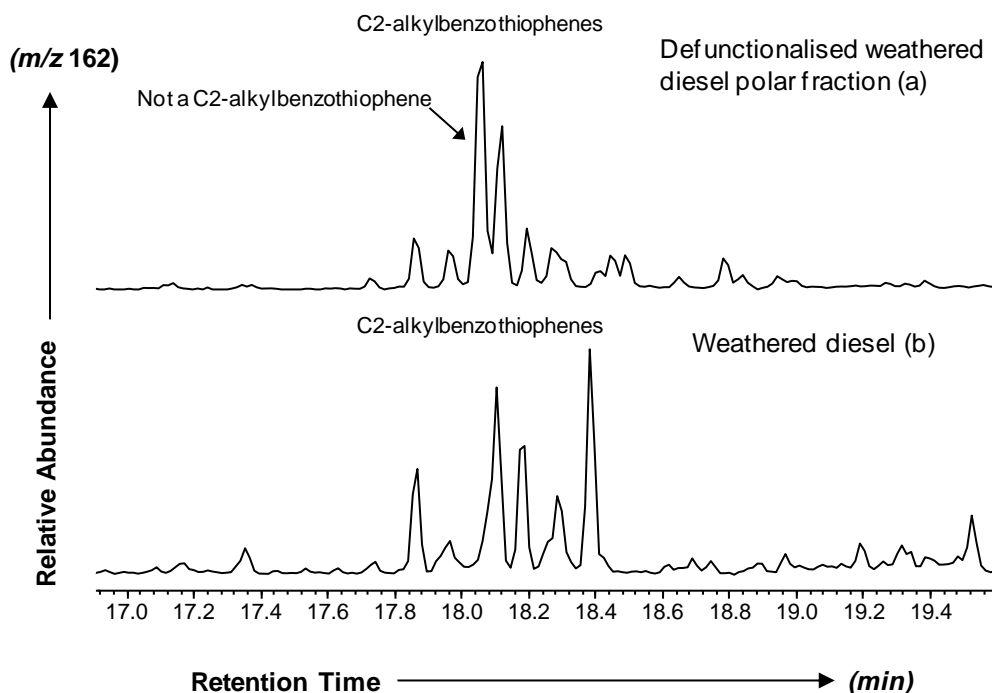


Figure 5.20 Partial ion chromatograms (m/z 168) showing the distribution of C2-alkylbenzothiophenes formed from defunctionalisation of a) weathered diesel polar fraction, and those present in b) weathered diesel analysed using GC-MS method 2.

5.5.2.1.2 Formation and accumulation of polar compounds in petroleum

Reaction products formed from the defunctionalisation of polar UCMs from petroleum can provide insights into the predominant compound types that occur in the UCM. Major reaction products formed relate to a range of biodegradation products derived from petroleum constituents. No reaction products were identified that relate to the easily biodegradable hydrocarbons (i.e. *n*-alkanes), suggesting these compounds (and their biodegradation products) have been completely removed from the weathered diesel and groundwater (e.g. mineralisation to CO_2). On the other hand, a series of reaction products likely formed from more biodegradation resistant compounds (alkylbenzenes, alkylnaphthalenes, alkylbiphenyls, alkyldibenzothiophenes and alkyladamantanes) were identified.

The presence of polar compounds in the reaction products formed from hydrocarbons that are very resistant to biodegradation (i.e. diamondoids) while other less resistant hydrocarbons are still present in significant abundances is unexpected, considering petroleum constituents are expected to be depleted in an apparent step-wise manner based on their relative susceptibilities to biodegradation (Kaplan *et al.*, 1996), possibly as a result of these compounds undergoing biodegradation at the same time at different rates. The diamondoids (adamantanes, diamantanes and triamantanes) are more resistant to biodegradation than most other hydrocarbons, and not expected to be significantly altered in crude oil until the *n*-alkanes, acyclic isoprenoids, dimethylnaphthalenes, methyl-, ethy- and dimethylbiphenyls, and bicyclic alkanes have been depleted, and the UCM significantly enhanced (Grice *et al.*, 2000). The presence of biodegradation products from highly biodegradation resistant hydrocarbons also suggests that other hydrocarbons with a similar resistance to biodegradation (hopanes, oleananes, steranes and diasteranes; Wei *et al.*, 2007) may also be biodegrading, including hydrocarbons that are usually present as a UCM and are generally considered recalcitrant (Blumer *et al.*, 1973; Gough and Rowland, 1990; Frysinger *et al.*, 2003).

The formation and accumulation of polar compounds formed from the biodegradation of highly biodegradation resistant compounds in comparable abundances to biodegradation products formed from compounds that are relatively susceptible to biodegradation may be accounted for by the mechanisms described in Chapter 4. For example, results from Chapter 4 showed that the TPH was heavily biodegraded at the uppermost region of a contaminated soil profile, which corresponded with an increased proportion of polar compounds. It is therefore possible that the biodegradation of these resistant compounds occurs at the top of the soil profile where the compounds most susceptible to biodegradation have been depleted, allowing for the biodegradation of these relatively biodegradation resistant compounds (i.e. diamondoids). This heavily biodegraded weathered diesel may then be recombined with the main NAPL body (along with biodegradation products

formed) through fluctuations in the water table and consequent movement of the NAPL.

The degradation of the entire petroleum is likely to have implications to monitored natural attenuation. Firstly, weathered petroleum is generally considered recalcitrant and not expected to be significantly attenuated through natural processes. Secondly, mass loss of petroleum through biodegradation has been estimated by observing changes in TPH concentrations relative to biodegradation resistant compounds, such as hopanes (Venosa *et al.*, 1997) and isoprenoids (Johnston *et al.*, 2007). This may underestimate mass loss if the entire petroleum is being biodegraded. The biodegradation of relatively resistant compounds shown in the present study suggests that mass loss of the entire weathered petroleum is likely to be occurring without significant changes to composition of weathered petroleum, and this would make it difficult to assess whether natural attenuation is taking place.

The very similar distributions of these reaction products from the weathered diesel polar fraction and groundwater TPH extracts also supports the suggestion in Chapter 4, that polar UCMs in groundwater largely consist of polar biodegradation products that form and accumulate in the weathered diesel. Therefore, it is suggested that the most frequently reported biodegradation products formed from petroleum (i.e. aromatic carboxylic acids formed in groundwater) account for only a very minor proportion of polar UCMs that give rise to high TPH concentrations in groundwater (Chapter 2).

5.5.2.1.3 Forensic applications for petroleum derived samples

The defunctionalisation technique may have potential to fingerprint and relate (or distinguish) petroleum (both NAPLs and organics measured as TPH) present in the environment as a polar UCM, which provides only very little characteristic information prior to defunctionalisation (e.g. GW-2 contained no identifiable polar compounds). The distributions of the major reaction products formed from the weathered diesel polar fraction and groundwater samples are remarkably similar, and these similarities have potential use in relating environmental samples that contain only UCMs of polar compounds. For example, although GW-1 and GW-2 are likely to have the same source because they are from the same site, these TPH extracts contained no identifiable hydrocarbons and a significantly different UCM profile. However, the defunctionalisation of the TPH extracts provided a hydrocarbon fingerprint that relates these samples to each other, as well as being very similar to the likely weathered diesel source.

Only one significant difference was shown in the reaction products formed from the weathered diesel polar fraction and groundwater TPH extracts, namely, the absence of alkylbenzothiophenes in the reaction products formed from the groundwater TPH extracts. This absence may be explained by the presence of microorganisms in the groundwater that have been shown to degrade thiophenes through the removal of the sulfur heteroatom (Xu *et al.*, 2006).

5.5.2.2 Polar UCM from NOM

A surface water sample sourced near a regional park surrounded by melaleuca trees (family Myrtaceae, angiosperms) was selected for defunctionalisation as it contained detectable TPH that consisted of a large UCM (Fig. 5.21). Anthropogenic inputs to this sample could not be discounted as the area receives stormwater drainage. However, the sample was obtained from a flooded area not connected to Herdsman Lake. Which

was heavily surrounded by vegetation. As a result, this sample was a selected as a good candidate to represent NOM and test the technique. The TPH measurement for the NOM sourced TPH extract was 13 mg/L, which was comparable to the TPH concentrations in the groundwater associated with weathered diesel contamination.

The NOM sourced TPH extract consisted of a large UCM with several small, resolved peaks in the early eluting part of the UCM (Fig. 5.21). Only one of these peaks could be identified, with eucalyptol tentatively identified based on its mass spectrum. Separation of the TPH extract into SAA (Fig. 5.21b) and POL fractions (Fig. 5.21c) showed that almost all of the TPH eluted in the polar fraction (over 99.9 % based on the TIC response).

Defunctionalisation of the polar fraction resulted in the formation of several resolvable peaks that were identified as aromatic hydrocarbons and heterocyclic polar compounds (Fig. 5.21d). The recovery of the products after defunctionalisation was less than 1 % based on the TIC response, which was significantly lower than the polar fractions from the weathered diesel (5 %) and related groundwater samples (GW-1, 9 % and GW-2, 7.5 %). As the NOM sourced sample was taken from a surface water location, the material that has been extracted in the TPH is likely to be polyfunctional. Therefore, these compounds may be more prone to polymerisation reactions, resulting in compounds too large for analysis using GC-techniques.

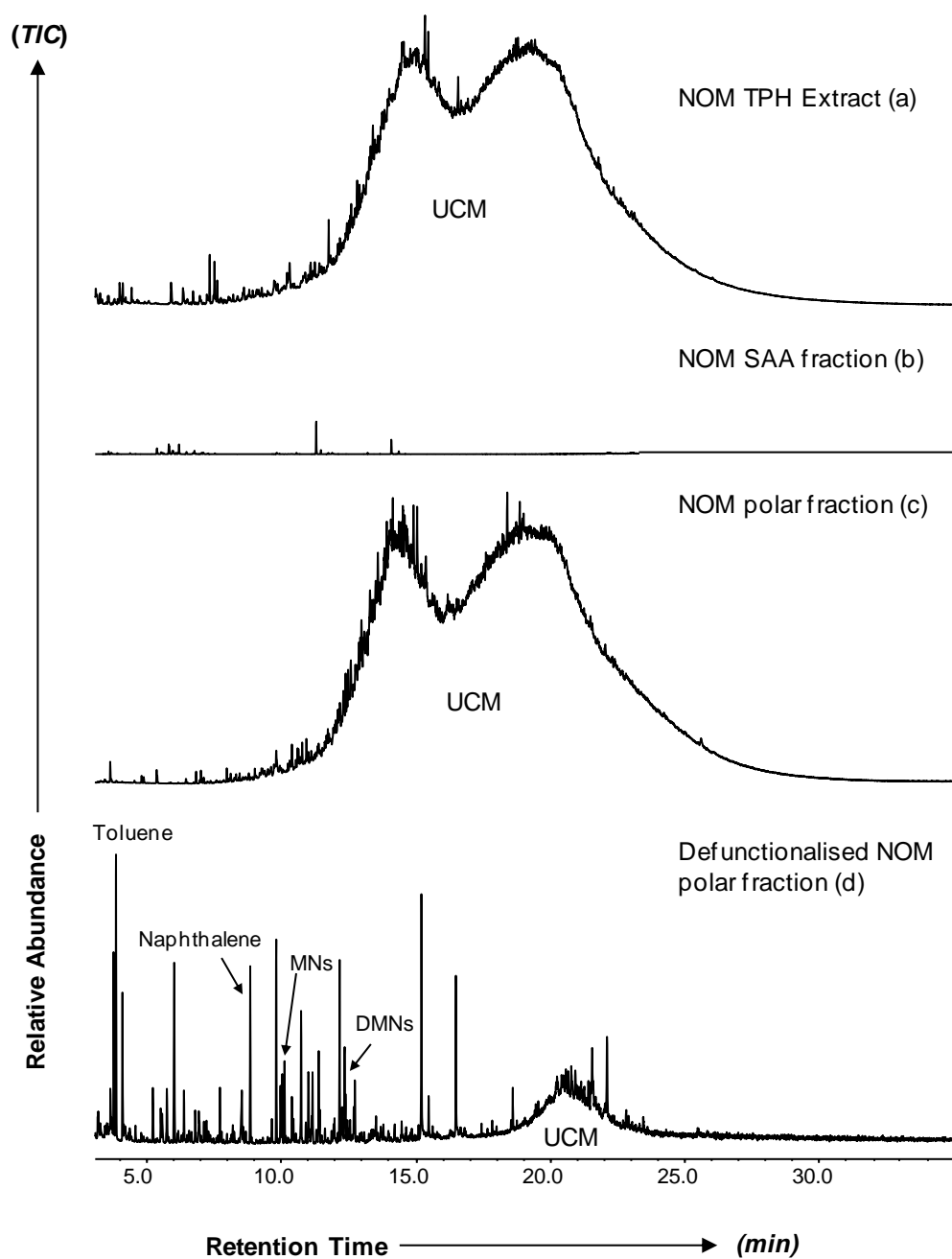


Figure 5.21 Total ion chromatograms (TIC) of a) NOM sourced TPH extract, b) NOM sourced TPH extract SAA fraction, c) NOM sourced TPH extract polar fraction, and d) defunctionalised NOM sourced TPH extract polar fraction analysed using GC/MS method 1. Chromatograms background subtracted to remove column bleed. MNs: methylnaphthalenes, DMNs; dimethylnaphthalenes.

Compound class characterisation of the defunctionalised NOM sourced TPH extract polar fraction provided aliphatic, aromatic and polar fractions (Table 5.9). No aliphatic components were observed, whereas aromatic components represented 41 % of the extracted organics, with polar components representing a slightly larger proportion (59 %).

Table 5.7 Proportions of aliphatic, aromatic and polar components in the defunctionalised groundwater TPH extract polar fractions

Compound class	Defunctionalised NOM products
Aliphatic fraction	0 %
Aromatic fraction	41 %
Polar fraction	59 %

5.5.2.2.1 NOM reaction products

Aromatic hydrocarbons

Aromatic hydrocarbons were the most abundant reaction products, and are clearly visible in the TIC for defunctionalised NOM products (Fig. 5.21d). These aromatic hydrocarbons include alkylbenzenes, alkylnaphthalenes, and alkylbiphenyls. No precursors to the range of compounds formed could be identified in the NOM TPH before defunctionalisation.

Alkylbenzenes

A range of alkylbenzenes were formed from the defunctionalisation of the NOM sourced polar fraction (Fig. 5.22). Distributions largely consisted of methyl isomers, with a limited number of alkylbenzenes with longer alkyl groups compared to the defunctionalised petroleum samples. This is most evident for the C4-alkylbenzenes where only few isomers have formed (Fig. 5.22c).

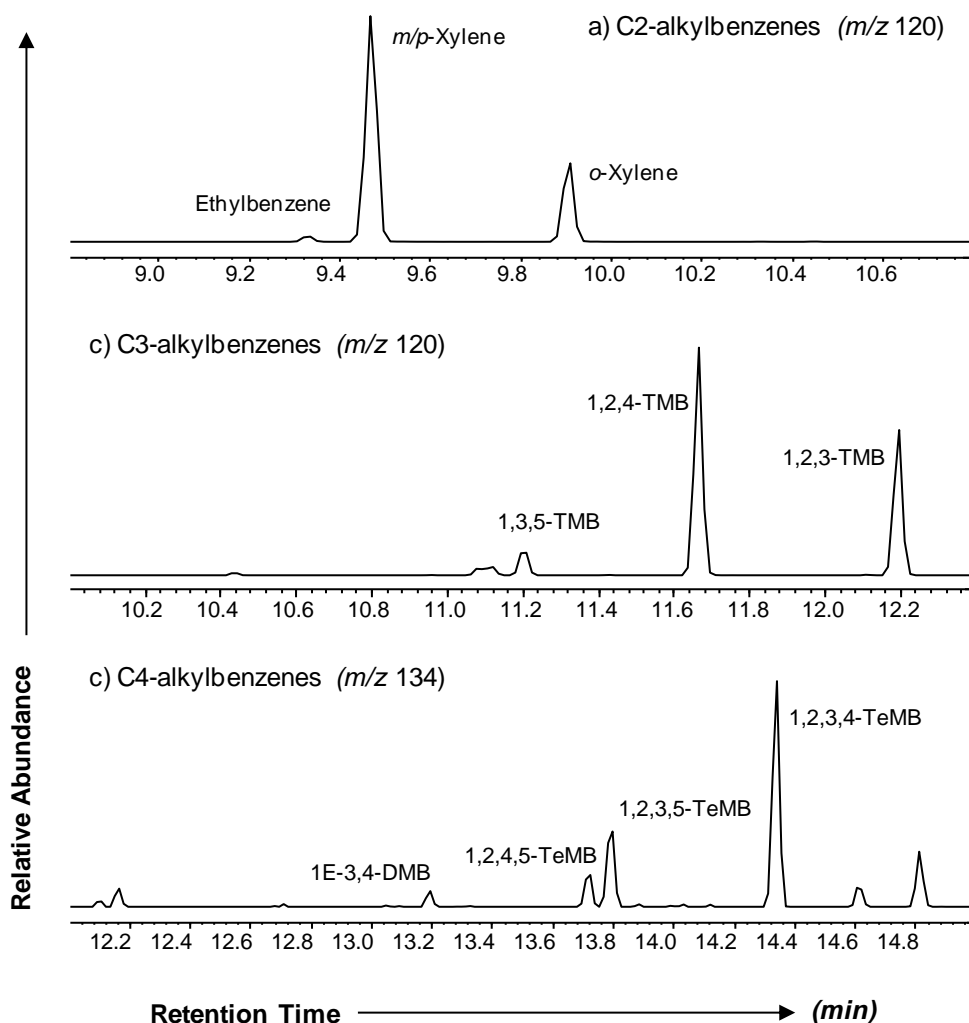


Figure 5.22 Partial ion chromatograms showing a) C2- (m/z 106), b) C3- (m/z 120), and c) C4-alkylbenzenes (m/z 134) formed from defunctionalisation of the NOM TPH extract analysed using GC-MS method 2. TMB: trimethylbenzene, E: ethyl, DMB: dimethylbenzene, TeMB: tetramethylbenzene.

The C3 and C4-alkylbenzenes formed from defunctionalisation (Fig. 5.22a,b) were dominated by 1,2,4-trimethylbenzene, 1,2,3-trimethylbenzene, 1,2,3,4-tetramethylbenzene with only one ethyl containing isomer identified. The C3- and C4-alkylbenzenes distributions were similar to those formed from the pyrolysis of resins, which are attributed to natural product structures (Hartgers *et al.*, 1994). For example, the most abundant trimethylbenzene (1,2,3- and 1,2,4-trimethylbenzenes; Fig. 5.22b) and tetramethylbenzene isomers (1,2,3,5- and 1,2,3,4-tetramethylbenzenes; Fig. 5.22c) are reported products from the flash pyrolysis of resins containing terpenoid structures,

such as plastoquinones (present in algae and cyanobacteria) and carotenoids (naturally occurring pigments; Hartgers *et al.* 1994). 1,2,3-Trimethylbenzene and 1,2,3,4-tetramethylbenzene would likely form from the defunctionalisation and aromatisation of a carotenoid, such as β -carotene (Fig. 5.23a) (1,2,3,4-tetramethylnaphthalene resulting from a 1,2- shift of a gem dimethyl group), whereas 1,2,4-trimethylbenzene would likely form from a plastoquinone structure (Fig. 5.23b) (Hartgers *et al.*, 1994).

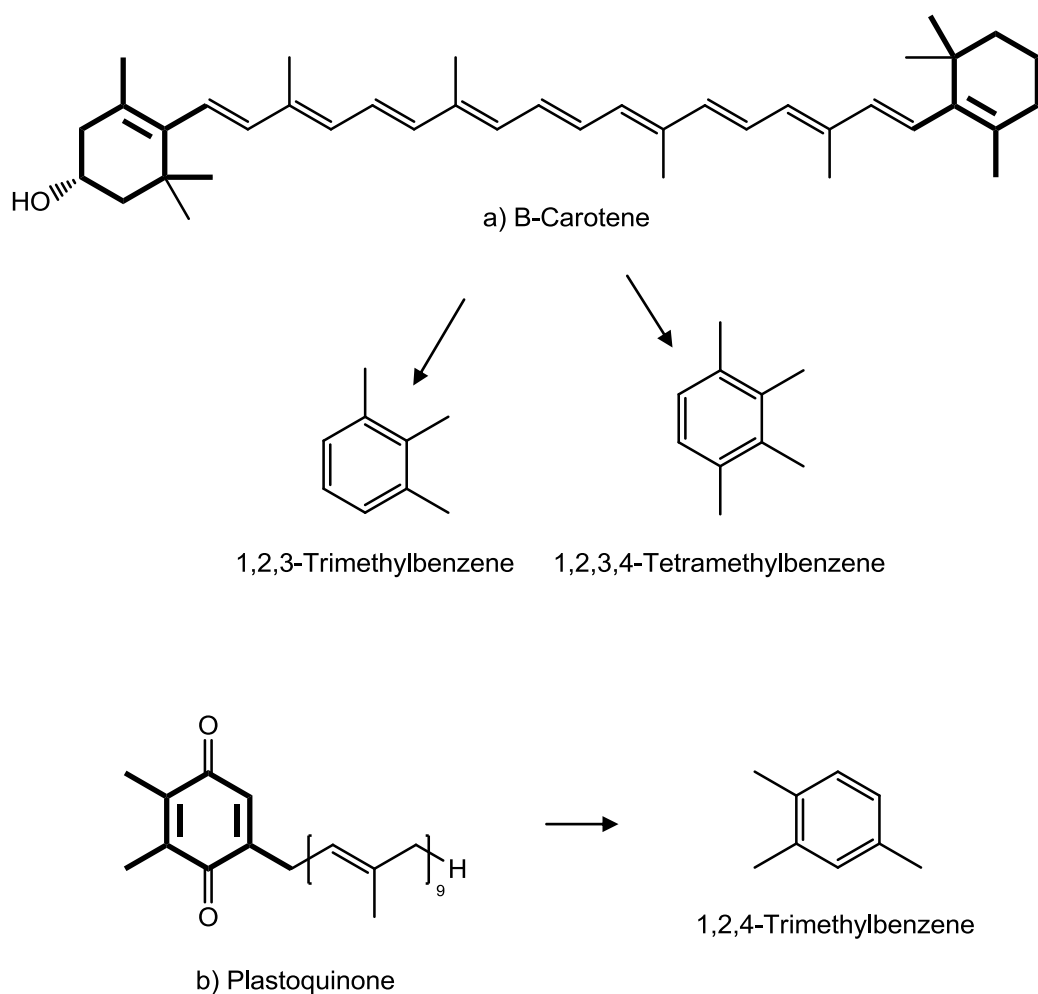


Figure 5.23 Proposed defunctionalisation and aromatisation of a) β -carotene to form 1,2,3-trimethylbenzene and 1,2,3,4-tetramethylbenzene and b) the proposed defunctionalisation and aromatisation of a plastoquinone to form 1,2,4-trimethylbenzene.

Other possible cyclic terpenoid sources of alkylbenzenes in the defunctionalisation products of the NOM sample include polycyclic compounds like abietic acid, which was used to optimise and test the defunctionalisation technique. Abietic acid formed 1,2,3-trimethylbenzene as a result of defunctionalisation, aromatisation and cleavage of cyclic ring system (Fig. 5.24), suggesting that the alkylbenzenes could form from a range cyclic natural products via a similar mechanism.

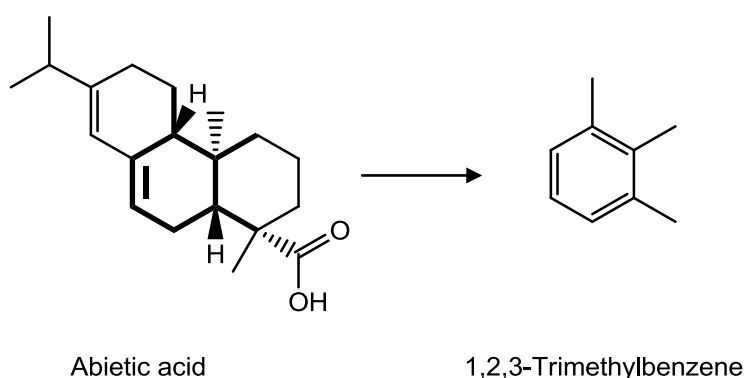


Figure 5.24 Proposed defunctionalisation and aromatisation of abietic acid to form 1,2,3-trimethylbenzene showing a potential pathway for the formation of trimethylbenzenes from natural products.

The cyclisation of branched terpenoids is another possible source for alkylbenzenes in NOM samples. The model compound phytanol (a branched terpenoid) underwent cyclisation, aromatisation and defunctionalisation to form *m/p*-xylenes as well as some specific alkylbenzene reaction products with isoprenoid alkyl groups. The absence of alkylbenzenes with isoprenoid alkyl groups in the reaction products of the NOM suggests these compound types were not abundant in the polar UCM from the NOM sample.

Alkylbenzenes can also form from the cyclisation, aromatisation, and defunctionalisation of less complex lipids containing straight chain structures, such as carboxylic acids (fatty acids) and alcohols (fatty alcohols), or molecules that contain these structures (e.g. triglycerides and phospholipids). However, *ortho*-substituted configurations were not dominant alkylbenzene reaction products, suggesting fatty acids and alcohols were also not abundant in the polar UCM. This is also supported by the absence of any

n-alkane aliphatic reaction products, which are also likely to form from the defunctionalisation of these compound types (Lang *et al.*, 2005).

Alkyl-naphthalenes

A range of alkyl-naphthalenes were formed from the polar UCM of the NOM sample (Fig. 5.25). Similar to the alkylbenzenes, the alkyl-naphthalenes formed consisted of predominantly methyl isomers, with only one alkyl-naphthalene (cadalene) formed with longer alkyl chains.

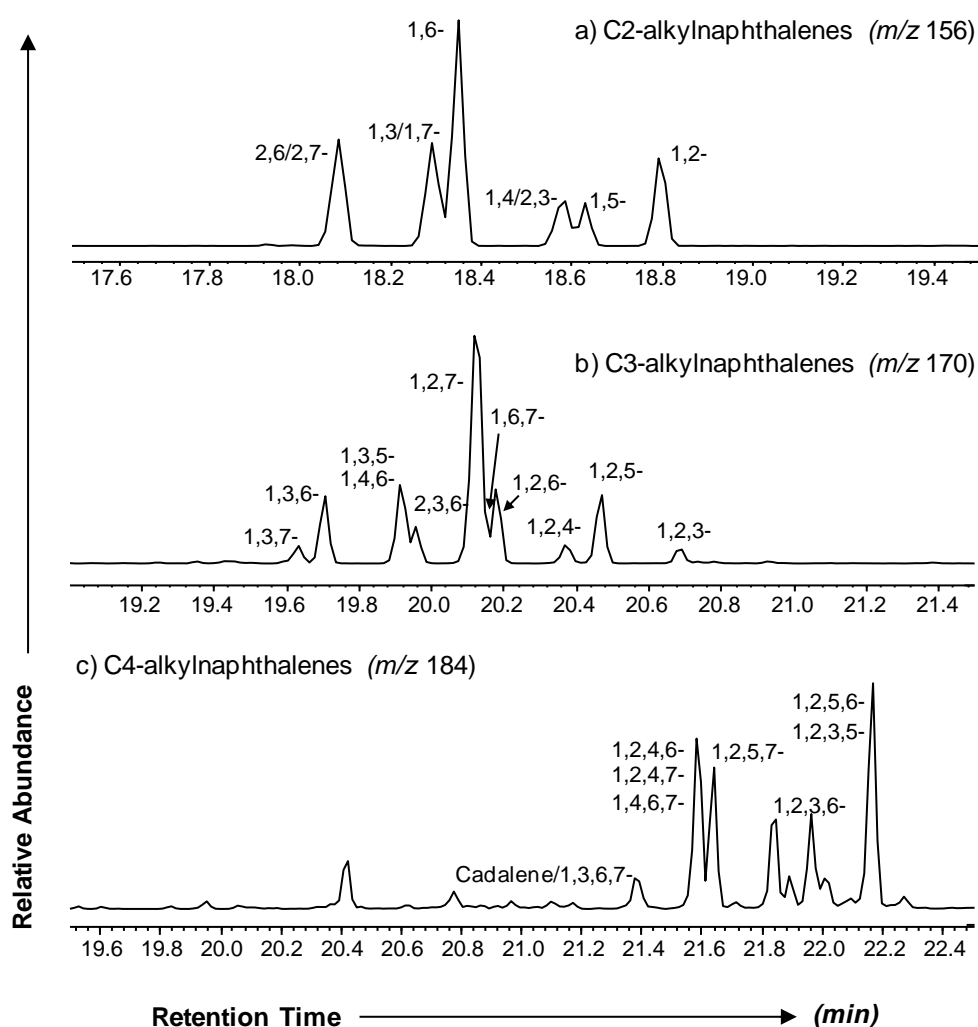


Figure 5.25 Partial ion chromatograms showing a) dimethyl- (m/z 156), b) trimethyl- (m/z 170) and c) tetramethylnaphthalenes (m/z 184) formed from defunctionalisation of the NOM TPH extract analysed using GC-MS method 2.

The distribution of C2-alkylnaphthalene isomers formed from the NOM sourced TPH is dominated by methyl isomers, with a high relative abundance of 1,6-dimethylnaphthalene (Fig. 5.25a). Enhanced relative abundances of this isomer in crude oil and sediments is suggested to be a result of the aromatisation of cyclic sesquiterpenenoid natural products derived from higher plants (van Aarssen *et al.*, 2000). Specifically, compounds containing the cadinane carbon skeleton, such as cadinene and cadinols derived from gymnosperms (Simoneit *et al.*, 1986), as well as resinous polymeric cadinene (polycadinene) produced by angiosperms (van Aarssen *et al.*, 1990; van Aarssen *et al.*, 1992). Therefore, the dominant 1,6-dimethylnaphthalene isomer formed in this study is likely a result of the defunctionalisation, aromatisation and cleavage of polar compounds containing the cadinane carbon skeleton (Fig. 5.26), indicating that some of the material present within the NOM sourced TPH extract is derived from terpenoids of higher land plants. This is also supported by the presence of cadalene in the reaction products (peak co-eluted with 1,3,6,7-tetramethylnaphthalene, but was identified due to the presence of its *m/z* 198 molecular ion; Fig. 5.25c).

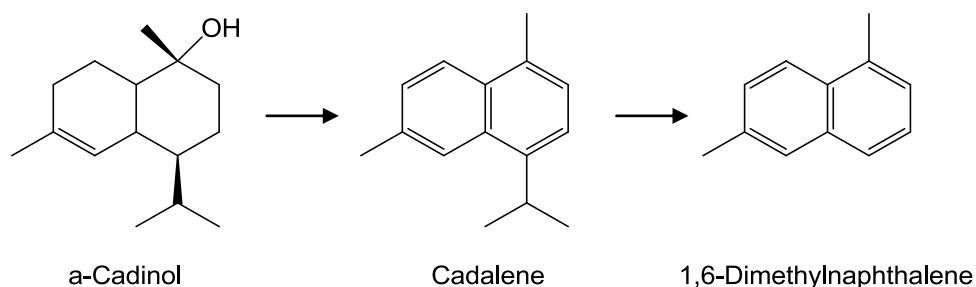


Figure 5.26 Proposed defunctionalisation and aromatisation of cadinol to form cadale, followed by cleavage to form 1,6-dimethylnaphthalene.

1,2,7-Trimethylnaphthalene was the dominant isomer present in the C3-alkylnaphthalenes (distinguished from 1,6,7-trimethylnaphthalene as the compounds only partially co-eluted) formed from the defunctionalisation of the NOM sourced TPH (Fig. 5.25b). Enhanced relative abundances of 1,2,7-trimethylnaphthalene in relatively immature sediments has been attributed to

oleanane triterpenoids present in angiosperms (Strachan *et al.*, 1988). Natural product chemists also produced 1,2,7-trimethylnaphthalene from the selenium dehydrogenation of the oleanane echinocystic acid (White and Noller, 1939), and the formation of 1,2,7-trimethylnaphthalene in the present study has likely occurred through a similar mechanism (Fig. 5.27). The presence of 1,2,7-trimethylnaphthalene provides further evidence that some of the material within the polar UCM from NOM is derived from higher land plants, such as the melaleuca trees present at the sampling location.

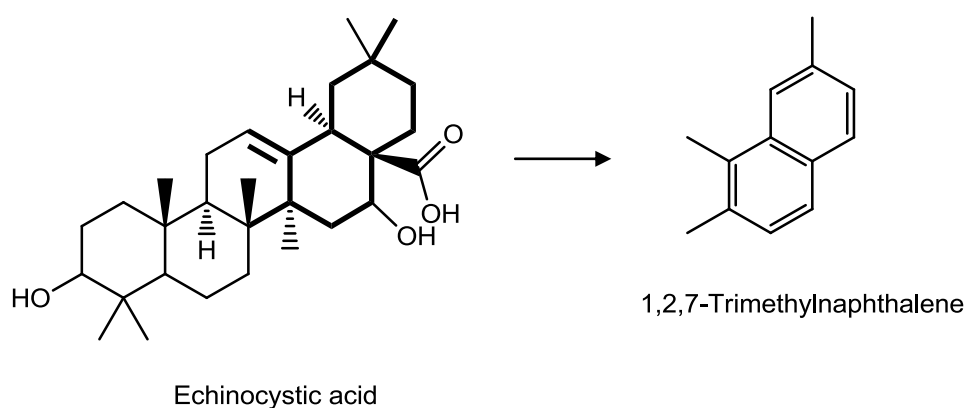


Figure 5.27 Proposed defunctionalisation and aromatisation of echinocystic acid to form 1,2,7-trimethylnaphthalene

Diphenylmethane and alkylbiphenyls

Diphenylmethane and alkylbiphenyls were formed from the defunctionalisation of the NOM sourced TPH polar fraction (Fig. 5.28). The alkylated biphenyls included C1- and C2-alkylbiphenyls and were predominantly methyl isomers. The distribution of methylbiphenyl isomers formed from the NOM sourced TPH is dominated by 3- and 4-methylbiphenyl, and a lower relative abundance of 2-methylbiphenyl (Fig. 5.28) and these compounds may form from a range of polar compounds from NOM. This includes lignin (Katayama *et al.*, 1988; Castro *et al.*, 2003) and tannins (Jacobs *et al.*, 1983) that are natural polymers containing the biphenyl carbon skeleton within their polymeric structure, and breakdown

products of lignin and tannins that may be incorporated in TPH extracts (Girard and Edelman, 1994).

Additional polar compounds that occur in nature that are known to cyclise and aromatise to form alkylbiphenyls include straight chain primary substituted polar compounds (i.e. fatty acids from algae). However, experiments performed with fatty acids under similar conditions to those performed here show these compounds typically afford alkylbiphenyls substituted at the 2- and 2,2'- positions (Bastow *et al.*, 2001; Lang *et al.*, 2005), which are not dominant within the alkylbiphenyls formed from the defunctionalisation of the NOM sourced TPH. This again indicates that fatty acids or alcohols are not abundant within the polar UCM from NOM, and that algae may not be a significant source of the NOM.

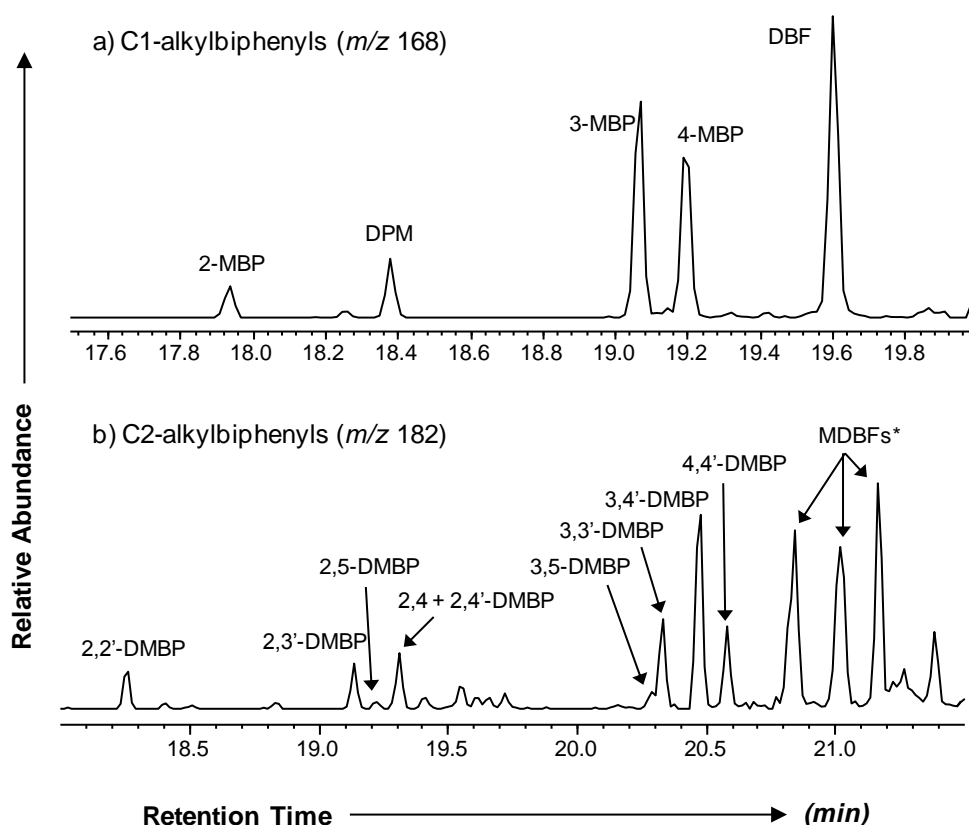


Figure 5.28 Partial ion chromatograms showing a) diphenylmethane, dibenzofuran and methylbiphenyls (m/z 168), and b) dimethylbiphenyls (m/z 182) formed from defunctionalisation of the NOM TPH extract analysed using GC-MS method 2. MBP: methylbiphenyl, DPM: diphenylmethane, DBF: dibenzofuran, DMBP: dimethylbiphenyl, MDBF: methyldibenzofuran, *structure not confirmed.

Polar compounds

Alkylbenzofurans and dimethyldibenzofurans were the major heterocyclic oxygen containing compounds identified in the defunctionalised NOM sourced TPH extract. This included a methylbenzofuran isomer, C2-alkylbenzofurans, dibenzofuran, and methyldibenzofurans. Configurations for these compounds could not be assigned.

Alkylbenzofurans

Methyldibenzofuran (Fig. 5.29a) isomers formed from the defunctionalisation of the NOM sourced TPH were identified in the reaction products. Natural precursors that may defunctionalise to form these compounds include polysaccharides and lignins, which have been suggested to be precursors to alkylbenzofurans identified in the pyrolysates of NOM (Sigleo, 1978; Berwick, 2009). Relatively simple polar compound structures may also undergo dehydrocyclisation to form benzofurans, as shown by the formation of benzofuran from the dehydrocyclisation of the model compound 2-ethylcyclohexanol. As a result, naturally occurring compounds with similar structures (e.g. terpenoids) can similarly undergo dehydrocyclisation to form alkylbenzofurans. Due to these varied potential sources of alkylbenzofurans, it is difficult to relate these reaction products to a specific source.

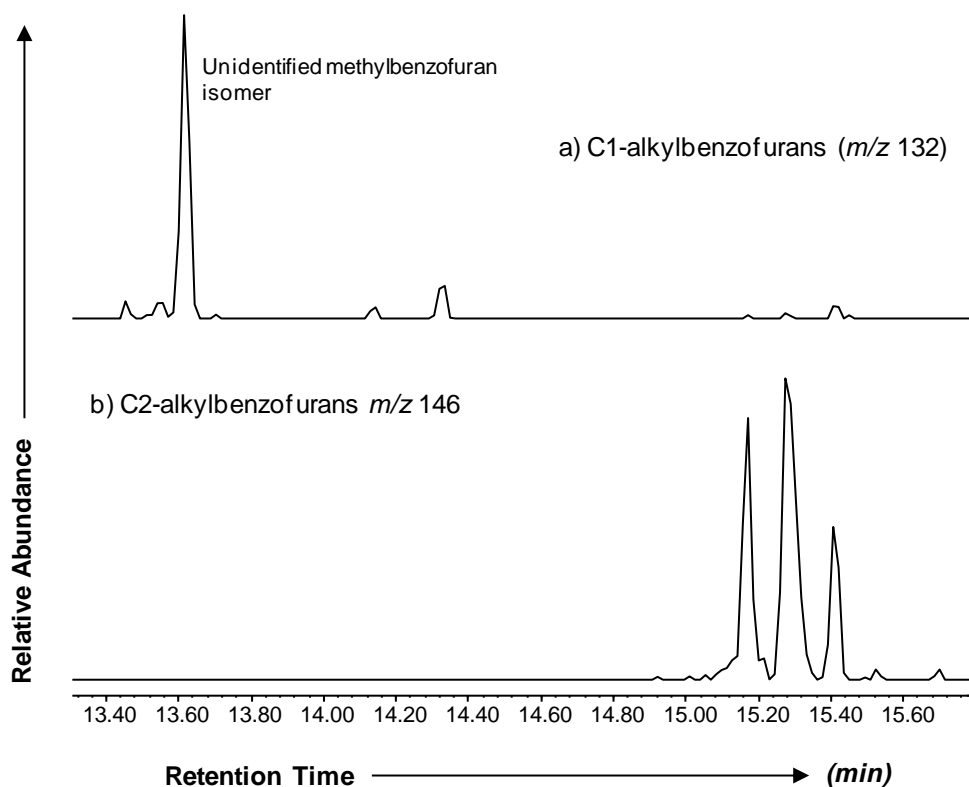


Figure 5.29 Partial ion chromatograms showing a) C1- (m/z 132), and b) C2-alkylbenzofurans (m/z 146) formed from defunctionalisation of the NOM TPH extract, analysed using GC-MS method 2.

Alkyldibenzofurans

Alkyldibenzofurans formed from the defunctionalisation of the NOM sourced TPH extract polar fraction (Fig. 5.28) may have the same natural product precursors as the biphenyls, considering they have the same carbon skeleton. For example, the dehydrocyclisation of alkylbiphenyls containing a hydroxyl group at the 2- position would result in the formation of dibenzofuran (Fig. 5.18). This includes a biphenyl structure present in some lignin (i.e. 2,2'-dihydroxy-3,3'-dimethoxy-5,5'-dicarboxybiphenyl) which also contains hydroxyl groups in the appropriate positions to undergo dehydrocyclisation to form the dibenzofuran structure (Katayama *et al.*, 1988).

5.5.2.3 Distinguishing petroleum and NOM polar UCMs

Reaction products formed from the defunctionalisation of petroleum and NOM derived polar UCMs can be used to distinguish their origins. The presence of diamondoid reaction products formed from the defunctionalisation of petroleum derived samples was the major difference observed in the defunctionalisation of polar UCMs associated with petroleum and NOM. Diamondoid carbon skeletons are ubiquitous in petroleum (Wingert, 1992; Wei *et al.*, 2007) and have no known natural precursors (formed through high temperature and pressure processes that occur during crude oil formation; Olah 1990). As a result, the presence of diamondoids as reaction products provides unequivocal evidence for petroleum derived polar compounds in the TPH extract UCMs. This is particularly useful in instances where TPH extracts contain no identifiable hydrocarbons or polar compounds and cannot be related to a specific source (i.e. GW-2 TPH extract and the TPH extract associated with NOM).

Alkylbenzenes, alkylnaphthalenes, alkylbiphenyls, alkylbenzofurans and alkyldibenzofurans were common to the defunctionalisation of the petroleum and NOM polar UCMs, though some differences were shown in the distributions of these compounds, and their use to distinguish the two sources may be more difficult. Differences in the alkylnaphthalenes in the NOM and petroleum reaction products included dominant alkylnaphthalene isomers formed from the defunctionalisation of NOM relating to natural products (e.g. dominant 1,6-dimethylnaphthalene and 1,2,7-trimethylnaphthalene isomers were related to higher land plant natural products). However, some care is needed when interpreting alkylbenzene and alkylnaphthalene defunctionalisation products where the associated petroleum release consists of immature crude oil, as natural product configurations may be dominant isomers prior to degradation.

Alkylbenzothiophenes were formed from the defunctionalisation of petroleum (weathered diesel polar fraction) but were not formed from the

defunctionalisation of NOM. The presence of these compounds in petroleum derived samples is dependant on the sulfur content of the petroleum. While not formed from the defunctionalisation of the NOM TPH extract, they have been produced from the pyrolysis of NOM (Saizjimenez and Deleeuw, 1986) and the flash pyrolysis of triglycerides and unsaturated fatty acids in the presence of elemental sulfur (Saizjimenez, 1994a). Although similar rearrangements are likely to be less significant using the dehydrogenation technique, the presence of alkyldibenzothiophenes may not be an absolute marker for the presence of petroleum. However, distributions of these compounds were similar to those present in petroleum, and would likely indicate a petroleum origin for the polar UCM.

Alkylbenzofuran and alkyldibenzofuran isomers were formed in significantly greater abundances from the defunctionalisation of NOM compared to the defunctionalisation of petroleum. Increased relative abundances of alkylbenzofurans from the defunctionalisation of the NOM TPH may relate to abundant natural precursors, namely polysaccharides (cellulose) and lignin (Sigleo, 1978). However, dibenzofurans are known constituents of petroleum, and their increased abundance alone is not adequate evidence for NOM and petroleum without relating dominant isomers to specific natural products.

5.6 Conclusions

A technique was developed that may allow the differentiation of petroleum and NOM polar UCMs present in TPH extracts based on differences in their carbon skeletons. The technique defunctionalises polar compounds present as a UCM into simpler compounds (predominantly hydrocarbons) with fewer possible isomers. Defunctionalisation and aromatisation was carried out using a Pt/C catalyst under optimum conditions (6 mg Pt/C per mg TPH extract at 280 °C for 16 hours). A range of polar model compounds were used to optimise and test the technique, and produced hydrocarbons and heterocyclic compounds without significant changes to their carbon skeletons. Environmental samples from petroleum and NOM sources

containing polar UCMs were used to evaluate the technique (some containing no identifiable hydrocarbons or polar compounds), which identified diamondoid carbon skeletons that are ubiquitous to petroleum and do not occur in nature. These diamondoid reaction products are ideal for indicating the presence of petroleum within TPH extracts that contain only unresolved polar compounds that offer no characteristic information.

The reaction products formed from the petroleum samples also provided insight into the formation, accumulation and composition of the polar UCMs from petroleum. The presence of degradation products of compounds that are very resistant (diamondoids) to biodegradation in polar UCMs from weathered petroleum suggested that natural attenuation may occur without any significant change to the composition of the NAPL. This also suggested that compounds that are considered to be recalcitrant (including the UCM) can be degraded through natural attenuation processes. Similarities in the distributions of reaction products formed from the weathered diesel polar fraction and groundwater TPH extracts suggested that the technique may also have forensic applications. This includes using the reaction products as a fingerprint to relate (or distinguish) polar UCMs present in groundwater, or relate polar compounds present in groundwater to their potential sources.

The defunctionalisation technique developed provides a simple, inexpensive characterisation tool that can be applied to TPH extracts after their initial measurement using GC-FID or GC-MS. Results from defunctionalisation can be used to distinguish polar UCMs from NOM and petroleum, and has potential forensic applications in fingerprinting polar UCMs in the environment. In addition, there is potential for compound specific isotope analyses to be performed on the defunctionalised products to give strong evidence for NOM and/or petroleum origins to TPH. This is made possible by the resolved nature of the defunctionalised products, whereas samples before defunctionalisation largely consisted of a UCM that prohibits compound specific isotope analyses.

Chapter 6

6 A simple and rapid screening tool for assessing the source of polar UCMs in the TPH from water samples based on TPH and TOC analyses

6.1 Abstract

A simple and rapid screening tool based on TOC and TPH measurements was developed to assess the likely source (NOM or petroleum) of TPH in water samples consisting of polar UCMs. The premise of this assessment was based on polar compounds from petroleum being more amenable to TPH analysis than NOM, which can contain compounds of high molecular weights and polarities. Water samples that were believed to be largely associated with NOM demonstrated a significantly lower percentage TPH relative to TOC (less than 2 %) compared to groundwater samples associated with weathered diesel (18 – 87 %). Based on these results, water samples containing a large polar UCM and a percentage TPH greater than 6 % may require further investigation to identify the likely source of the TPH. These further investigations include the identification of polar compounds derived from petroleum using GC-MS techniques (Chapter 4) or the defunctionalisation of polar UCMs (Chapter 5).

6.2 Introduction

The sources of polar UCMs in TPH analyses can be difficult to establish (Girard & Edelman, 1994; Chapter 2; Chapter 3). However, the application of simple analyses such as TOC and TPH may help assess the likely sources of polar UCMs present in TPH analyses.

Polar UCMs present in environmental water samples associated with petroleum contamination have been identified as biodegradation products of petroleum (Chapter 4) and NOM (Girard & Edelman, 1994). The incorporation of these compounds into TPH analyses is limited by their polarity and molecular weight, as compounds that are too polar and high in molecular weight are not amenable for detection in TPH analyses that use GC techniques for detection (typical TPH range of C6 to C36) (TPHCWG, 1998).

The types of NOM that can be incorporated in typical TPH analyses of water are relatively unexplored. Girard and Edelman (1994) suggested that groundwater TPH downgradient from a wood waste disposal site was a result of breakdown products of lignin and tannins that were derived from NOM. In general, the composition of NOM is diverse, with a wide range of polarities and molecular weights (Aiken *et al.*, 1985). A significant proportion of NOM includes relatively high molecular weight polyfunctional compounds such as biopolymers (e.g. lignin and tannins) and humic substances (humic substances typically accounting for 50-75% of NOM in natural waters: Aiken *et al.* 1985). These compounds are generally not GC-amenable without pyrolytic or chemical cleavages prior to analysis (Christy *et al.*, 1999; Schulten and Gleixner, 1999; Leenheer *et al.*, 2003).

6.3 Scope of study

This study is aimed at developing a simple screening method to identify the likely source (NOM or petroleum) of polar UCMs present in TPH analyses from water samples. The proportions of TPH and TOC present in water samples was used to indicate the likely source of the polar UCMs, based on the higher proportion of non-GC amenable compounds present in NOM derived samples.

6.4 Experimental

6.4.1 Sample details

6.4.1.1 Petroleum

Groundwater samples associated with weathered diesel used in Chapter 5 for defunctionalisation (GW-1 and GW-2) were also used to test the technique (details provided in section 5.3.3). Weathered diesel-1 (used in Chapters 3, 4 and 5) was used for water equilibrations and was collected from bailing well installations and obtained from the same site as the groundwater samples.

6.4.1.2 NOM

NOM samples were collected from areas that are not likely to contain significant levels of petroleum contamination, though in some cases may contain anthropogenic inputs (Fig. 6.1). Groundwater samples (~500 ml) associated with NOM were collected from bailing well installations located in Western Australia, with no likely anthropogenic inputs. Surface water (~500 ml) samples (SW1-6) were collected from a range of water catchments in Southern River and Ellenbrook, Western Australia. Due to their agricultural and urban surroundings to these catchments, anthropogenic inputs are likely to be present. However, no significant petroleum input is likely to contribute to the organic matter. The natural surface water sample used in Chapter 5 for defunctionalisation (SW-7) was also used to test the technique (sampling details provided in section 5.3.4).

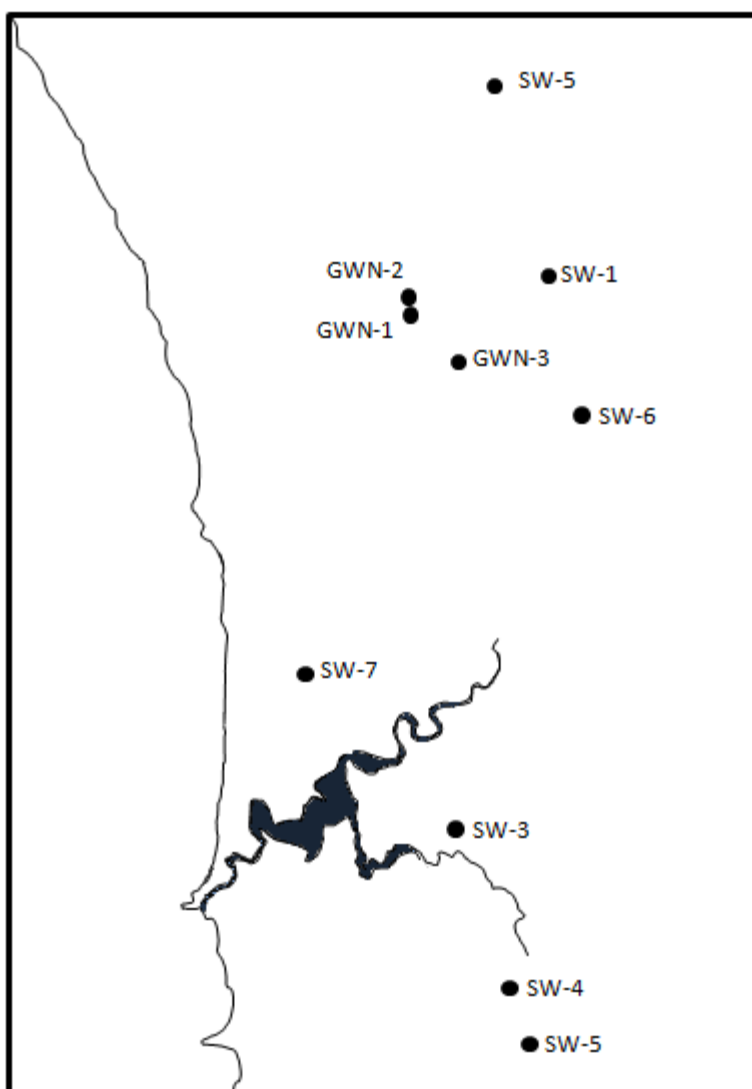


Figure 6.1 Site locations for groundwater (GWN) and surface water (SW) associated with NOM.

6.4.2 Equilibration experiments

Weathered diesel equilibration experiments were carried out in duplicate with an equilibration time of 96 hrs, along with duplicate procedural blanks. Details of this procedure can be found in section 3.4.2.

6.4.3 Total petroleum hydrocarbons (TPH)

TPH extraction and quantification for both petroleum and NOM associated water samples was performed using the procedure outlined in section 4.4.3.2 for groundwater associated with petroleum.

6.4.4 Analysis by Gas Chromatography Mass Spectrometry (GC-MS)

GC-MS analysis was performed using the method described in section 4.4.4.

6.4.5 Total organic carbon (TOC)

TOC measurements for environmental water samples were obtained with a Shimadzu TOC-VCPH. TOC was measured by non-purgeable organic carbon combustion catalytic oxidation with non-dispersive infrared (NDIR) detection. Pre-acidified samples were purged with nitrogen gas for 3 minutes to remove carbonates prior to quadruplicate analysis (injection volume of 2.5 ml). TOC was measured against potassium hydrogen phthalate calibration standards.

6.5 Results and discussion

Water samples associated with weathered diesel and a range of coloured water samples associated with NOM were selected for the study. TOC and TPH concentrations for the samples are shown in Table 6.1, as well as the percentage of TPH relative to TOC.

6.5.1 Water samples associated with weathered diesel

Water samples associated with weathered diesel included water equilibrated with weathered diesel and groundwater associated with weathered diesel (Table 6.1). The water equilibrated with weathered diesel contained a higher percentage TPH (87 %) compared to the groundwater samples associated with weathered diesel (18 – 28 %). Components that were not captured in the

TPH analysis for the equilibrated water but were measured in the TOC may include polyfunctional degradation products or accumulated biomass derived from the microorganisms that biodegrade petroleum (Riis *et al.*, 1996). The lower percentage TPH found for the groundwater samples compared to the equilibrated water may be a result of polar components that partition from the weathered diesel undergoing further biodegradation in the groundwater and forming more polar (i.e. polyfunctional) compounds that are not GC-amenable. Alternatively, the increased TOC relative to TPH may be a result of NOM components in the groundwater that are not amenable to GC.

Table 6.1 TOC and TPH concentrations, and % TPH (100 x TPH/TOC) for water samples associated with petroleum and NOM sources.

Sample	TOC (mg/L)	TPH C10-36 (mg/L)	%TPH
<i>Water equilibrated with weathered diesel</i>			
WDEQ	126	110	87
<i>Groundwater associated with weathered diesel</i>			
GW-1	190	35	18
GW-2	64	18	28
<i>Groundwater associated with NOM</i>			
GWN-1	54	<0.5	<1
GWN-2	53	2.5	5
GWN-3	60	<0.5	<1
<i>Surface water associated with NOM</i>			
SW-1	50	<0.5	<1
SW-2	21	<0.5	<1
SW-3	31	<0.5	<2
SW-4	3.9	<0.5	<13
SW-5	62	<0.5	<1
SW-6	41	<0.5	<1
SW-7	820	13	2

6.5.2 Water samples associated with NOM

Groundwater samples associated with NOM used in the study were obtained from areas vegetated with banksia (GWN-1-2) and pine (GWN-3) located in areas away from human activities. Surface water samples (SW-1-6) were obtained from water catchments located in areas that were not likely to be contaminated by petroleum. The natural surface water sample (SW-7, also used in Chapter 5) was obtained near a regional park surrounded by melaleuca trees (family Myrtaceae, angiosperms).

The percentage TPH for samples associated with NOM was generally low (less than 2 %) with the exception of two samples. The surface water sample, SW-4 had a higher percentage TPH (13 %) which was a result of the low TOC (3.9 mg/L) measured. The groundwater sample (GWN-2; Fig. 6.2) also contained a higher percentage TPH (5 %) which due to a series of peaks that included compounds identified as plasticisers. Sources of these plasticisers include the monitoring bore casing or some other plastic source that were not likely present in the groundwater (i.e. artefacts of sampling and analysis). The percentage TPH for this sample was therefore not representative of NOM in the groundwater.

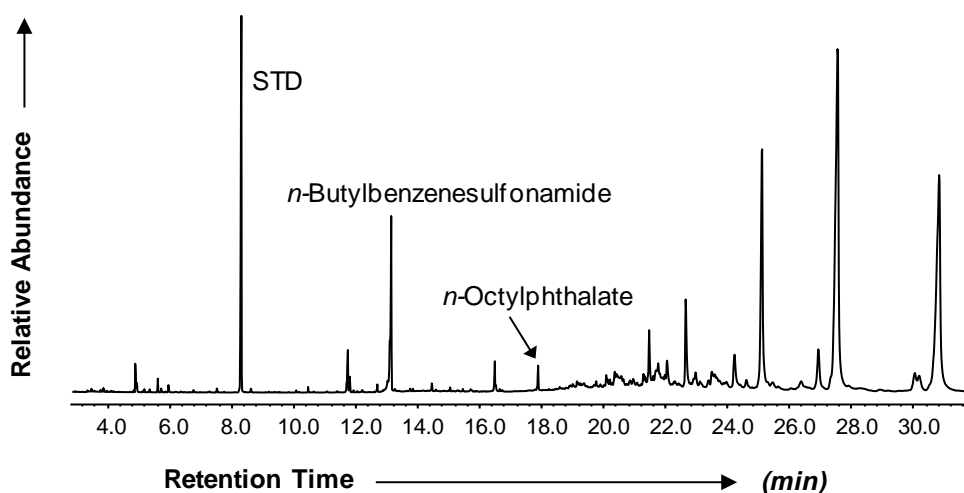


Figure 6.2 Total ion chromatogram (TIC) for GWN-2 TPH extract. ISTD: Internal standard.

The low proportion, or absence of TPH in the water samples associated with NOM is not surprising considering a significant proportion (50 – 70 %) of NOM in natural waters consists of high molecular weight humic substances (Aiken, 1985). Other major NOM types such as carbohydrates (polysaccharides, monosaccharides), proteins, peptides, lipids, amino sugars, lignins and tannins also have relatively high molecular weights and/or polarities (Gadel and Bruchet, 1987; Kordel *et al.*, 1997; Lehtonen *et al.*, 2001; Lee, 2003) that are not GC-amenable, and not likely to be captured in TPH analyses.

6.5.3 A screening tool for assessing the source of polar UCMs in TPH analyses

The percentage TPH in NOM related samples was generally lower (less than 2 % for all samples except those with a low TOC, and plasticiser interferences) than the samples associated with weathered petroleum (18 - 87 %). Therefore, based on these results a percentage TPH greater than 6 % (a value approximately three times less than the lowest petroleum sourced sample and three times larger than the highest NOM sourced sample) may suggest a possible petroleum source and require more detailed analyses to assess the likely source of the TPH. This includes the techniques outlined in Chapter 4 (identification of individual polar compounds using GC-MS) and Chapter 5 (differentiation of polar UCMs based on differences in their carbon skeletons).

6.6 Conclusions

A simple and rapid screening tool (percentage TPH relative to TOC) was developed to assess the likely source (NOM or petroleum) in TPH present as polar UCMs in water samples. Groundwater samples associated with NOM generally had a low percentage TPH (less than 2 %), whereas TPH containing polar UCMs associated with weathered diesel had a higher percentage TPH (18 – 87 %). This higher percentage TPH in samples

associated with petroleum compared to NOM could be used to predict a petroleum source for TPH present as a polar UCM. Based on the results of this study, further investigation may be required where the percentage TPH is greater than 6 %. This may include the identification of polar compounds present in the polar UCM using GC-MS or defunctionalisation of the UCM to produce a range of hydrocarbons that can be related to petroleum and/or NOM based on their carbon skeletons (Chapters 4 and 5 of this thesis respectively).

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